UNIVERSITA' VITA-SALUTE SAN RAFFAELE

CORSO DI DOTTORATO DI RICERCA
INTERNAZIONALE
IN MEDICINA MOLECOLARE

CURRICULUM IN BASIC AND APPLIED IMMUNOLOGY
AND ONCOLOGY

DoS: Prof. Angelo A. Manfredi

Second Supervisor: Prof.ssa Francesca Granucci

Multidirectional dysfunction of the immune response in patients with systemic lupus erythematosus

Tesi di DOTTORATO di RICERCA di:

Dr. Giuseppe Alvise Ramirez

Matr.: 013900

Ciclo di dottorato: XXXIV

SSD: MED/16, MED/09

Anno Accademico 2020/2021

CONSULTAZIONE TESI DI DOTTORATO DI RICERCA

Il/la sottoscritto/I	Giuseppe Alvise Ramirez
Matricola / registration number	013900
Nato a/ born at	Venezia
il/on	04/02/1987

autore della tesi di Dottorato di ricerca dal titolo / author of the PhD Thesis titled

Multidirectional dysfunction of the immune response in patients with systemic lupus erythematosus

☒ AUTORIZZA la Consultazione della tesi / AUTHORIZES the public release of the thesis

□ NON AUTORIZZA la Consultazione della tesi per …… mesi /DOES NOT AUTHORIZE the public release of the thesis for …… months a partire dalla data di conseguimento del titolo e precisamente / from the PhD thesis date, specifically Dal / from ……/……/…… Al / to ……/……/……

Poiché /because:

□ l'intera ricerca o parti di essa sono potenzialmente soggette a brevettabilità/ The whole project or part of it might be subject to patentability;

□ ci sono parti di tesi che sono già state sottoposte a un editore o sono in attesa di pubblicazione/ Parts of the thesis have been or are being submitted to a publisher or are in press;

□ la tesi è finanziata da enti esterni che vantano dei diritti su di esse e sulla loro pubblicazione/ the thesis project is financed by external bodies that have rights over it and on its publication.

E' fatto divieto di riprodurre, in tutto o in parte, quanto in essa contenuto / Copyright the contents of the thesis in whole or in part is forbidden.

Data /Date 25/03/2022 Firma /Signature Giuseppe Alvise Ramirez

DECLARATION

This thesis has been composed by myself and has not been used in any previous application for a degree. Throughout the text I use both 'I' and 'We' interchangeably.

All the results presented here were obtained by myself, except for:

1) *Human Leukocyte Antigen genotyping (*Figure 6: high-resolution HLA-DRB1 allele frequencies*) were performed in collaboration with Dr Benedetta Allegra Mazzi, Immunogenetics Laboratory, HLA & Chimerism, Department of Immunohematology & Blood Transfusion, IRCCS Ospedale San Raffaele Milan, Italy*

2) *Part of DNA extraction (*Figure 6: high-resolution HLA-DRB1 allele frequencies*) was performed in collaboration with Dr Lorena Citterio, Dr Laura Zagato, and Dr Elisabetta Messaggio, Research Unit on Genomics of Renal Diseases and Hypertension, IRCCS Ospedale San Raffaele Milan, Italy.*

*3) Part of the clinical data regarding patients' allergy history (*Figure 2: timing of onset of allergy in relation to SLE onset, Figure 3: onset of the first and second allergic reactions in patients with SLE, Table 3: allergy features in patients with SLE*) was collected by Dr. Andrea Sorce, Università Vita-Salute San Raffaele, Milan, Italy.*

All sources of information are acknowledged by means of reference.

ACKNOWLEDGEMENTS

I would have not been able to perform this work without the guidance of my DoS, Prof. Angelo Manfredi, who had a fundamental role in helping me with the design of the study and in supervising its progresses during these years, besides covering the costs of the majority of the experiments. I am also grateful to Prof. Chiara Bonini and her lab for fundamental advice regarding the general design of the study and for letting me the opportunity to perform most of the experiments in her Unit. I am also indebted with Dr Elena Tassi and Dr Maddalena Noviello from Prof. Bonini's lab for their special help in refining, enriching and strengthening the experimental design of my project and for invaluable practical hints that made it possible to translate it into reality. I thank my second supervisor Prof. Francesca Granucci for scientific counselling. I am also grateful to Dr Zulma Magnani, Dr Francesco Manfredi, Dr Laura Falcone, Dr Rita El Khoury, Dr Beatrice Cianciotti, Dr Clara Sciorati, Dr Annalisa Capobianco, Dr Norma Maugeri, Dr Antonella Monno, Dr Valeria Beretta, Dr Veronica Valtolina, Dr Matteo Doglio for their scientific and practical help with multiple aspects of the experiments. I would also like to thank Dr Francesco Manfredi and Dr Claudia De Lalla for having kindly provided me with multiple reagents. Although I was unfortunately unable to purchase reagents from the Benaroya Research Institute (BRI), Washington, USA, due to bureaucratic issues within my Institution, I must thank Dr Eddie James and Prof. Bill Kwok from the BRI for spending their time in counselling me about potential candidate epitopes for T-cell phenotypes. Furthermore, I acknowledge the inspirational value of their longstanding work on this subject to design my own project. I am also grateful to Proimmune® for further counselling on MHC-peptide combinations.

I must also acknowledge with gratitude the work of Dr Benedetta Mazzi and her team (see above), who performed Human Leukocyte Antigen (HLA) genotyping and the help of Dr Laura Zagato, Dr Lorena Citterio, and Dr Elisabetta Messaggio from the Research Unit on Genomics of Renal Diseases and Hypertension of IRCCS Ospedale San Raffaele for performing part of the DNA extraction procedures needed before HLA genotyping.

Clinical data and blood samples had started being collected before the beginning of this project as part of a greater collaborative effort to dissect the pathophysiological bases of immune-mediated diseases. To this regard, I am pleased to acknowledge the work of

my former and present colleagues in the Lupus Clinic of the IRCCS Ospedale San Raffaele, Dr Enrica Bozzolo, Dr Luca Moroni, Dr Valentina Canti and the fundamental support of Prof. Lorenzo Dagna, head of the Unit of Immunology, Rheumatology, Allergy and Rare Diseases (UniRAR) of the same Institution. I would also express my gratitude Dr Mona-Rita Yacoub, Dr Giselda Colombo (from the Allergy team of UniRAR), Dr Enrico Tombetti, Dr Elena Baldissera, Prof. Patrizia Rovere-Querini and Dr Rebecca De Lorenzo for their scientific and clinical help and for providing me with part of the samples employed in this study. I am also grateful to all residents and students who contributed to clinical activities and data collection. To this regard, I must dedicate a special mention to Dr Andrea Sorce (Università Vita-Salute San Raffaele) and Dr Francesca Cicero (Università di Messina).

I would also gratefully acknowledge the work of the San Raffaele Biological Resources Centre, with special thanks to Dr Cristina Tresoldi, Dr Elisa Cantarelli, Dr. Serenesse Tomasi and Dr Michela Grossi.

ABSTRACT

Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease characterised by protean clinical manifestations and a multifaceted pathogenic background. Allergic reactions and infectious events complicate the course of SLE, but their reciprocal correlations are poorly understood. Availability of accurate tools to stratify patients with homogeneous endo/phenotypes and guide personalised treatments is still an unmet need. Despite the potential pathogenic role of T cells in SLE, little is known about the quantitative and qualitative features of antigen-specific T cell responses. Based on clinical data demonstrating a tripartite association between disease flares, allergic and infectious events, a multi-step experimental plan was designed to seek and characterise antigen-specific T cells recognising histone-, Epstein-Barr virus (EBV)- and penicilloylated albumin-derived peptides in patients with SLE in comparison to patients with Takayasu's arteritis and healthy controls. Genetic studies confirmed that human leukocyte antigen (HLA)-DRB1*03:01 is a risk factor for SLE and revealed novel associations among DRB1*11:01, allergic and infectious events, and between DRB1*07:01 and infection protection. Stem-cell memory T cells (T_{SCM}) were expanded in patients with SLE possibly accounting for persisting inflammation. Using direct *ex vivo* visualisation of antigen-specific T cells stained with class II HLA tetramers through flow cytometry, histone-specific CD4+ T cells were selectively detected in patients with SLE and their accumulation in the peripheral blood associated with the presence of anti-DNA antibodies. Penicilloylated albumin-specific T cells identified patients with beta-lactam allergy. EBV-specific cells were detected as expected in patients and controls. Variations in the size of the three types of antigen-specific T cell populations were reciprocally correlated and cytokine responses to isolated epitopes revealed activation of multiple inflammatory pathways suggesting cross-contamination between antimicrobial, allergic and autoreactive responses. Histone-specific and EBV-specific effector memory T cells and T regulatory cells decreased during SLE flares, possibly reflecting peripheralization into target tissues and defective anti-inflammatory responses. EBV-specific T_{SCM} also decreased and EBV-induced cytokine responses were impaired during active phases of the disease possibly indicating mis-differentiation of precursors, and ineffective antiviral responses. These data support the existence of a multidirectional dysfunction of the

immune response, possibly traceable and targetable through T cell responses, as a pathophysiological and clinical hallmark of SLE.

TABLE OF CONTENTS

ACRONYMS AND ABBREVIATIONS

Acronym/abbreviation	Description
AAV	ANCA-associated vasculitides
aB2GPI	Anti-beta 2 glycoprotein I antibodies
aCL	Anti-cardiolipin antibodies
ACR	American College of Rheumatology
ADNA	Anti-(double-stranded)DNA antibodies
ANA	Anti-nuclear antibodies
ANCA	Anti-neutrophil cytoplasmic antibodies
APC	Allophycocyanin
aPL	Anti-phospholipid antibodies
APRIL	a proliferation inducing ligand (APRIL)
APS	Antiphospholipid syndrome
aRNP	Anti-ribonucleoprotein antibodies
aSm	Anti-Smith antigen antibodies
aSSA	Anti-SSA antibodies
aSSB	Anti-SSB antibodies
AZA	Azathioprine
BAFF	B cell activating factor
BILAG	British Isles Lupus Assessment Group
BCR	B cell receptor
CI	Confidence interval
CMV	Cytomegalovirus
COVID-19	SARS-CoV-2-related disease
DMSO	Di-methyl-sulfoxide
DVT	Deep vein thrombosis
ECLAM	European Consensus Lupus Activity Measure
GCA	Giant cell arteritis
HLA	Human leukocyte antigen
HR	Hazard ratio
IFNα	Interferon alpha
IFNγ	Interferon gamma

IL1, 2, 3…N	Interleukin 1, 2, 3…N
IQR	Interquartile range
IRF	Interferon regulatory factors
LAC	Lupus anticoagulant
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
MTX	Methotrexate
NET	Neutrophil extracellular trap
OR	Odds ratio
PBS	Phosphate buffered saline
PDN	Prednisone
PE	Phycoerythrin
PerCP	Peridinin-chlorophyll-protein
PHA	Phytohaemagglutinin
PRR	Pattern recognition receptors
PTPN22	Protein tyrosine phosphatase N22
RR	Relative risk
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SDI	SLICC/ACR damage index
SLE	Systemic lupus erythematosus
SLICC	SLE International Collaborating Clinics
TAK	Takayasu’s arteritis
TCR	T cell receptor
TGFβ	Transfoming growth factor beta
TLR	Toll-like receptor

LIST OF FIGURES AND TABLES

Figures

Tables

INTRODUCTION

General premise

Systemic lupus erythematosus (SLE) is a complex inflammatory disease characterised by multi-organ damage and broad inter- and intraindividual variability both in terms of pathophysiology and clinical phenotypes (Arnaud & Tektonidou, 2020, Rahman & Isenberg, 2008). Autoimmune manifestations are usually regarded as the hallmark of the disease, whereas the potential role of coexisting immune dysfunction in terms of susceptibility to infections and allergy has less been explored. Despite being conventionally classified as a rare disease (OrphaNet code 536), SLE can affect up to 1.6 persons per 1,000 inhabitants (Danchenko, Satia et al., 2006) and, along with other immune-mediated disorders (Huscher, Merkesdal et al., 2006), might have a significant social impact due to disease- and drug-related morbidity (Scofield, Reinlib et al., 2008). Human and economic costs of this disease are in fact particularly high, especially since SLE preferentially affect young people, and specifically women of childbearing age. Furthermore, disease-related morbidity and costs increase with disease duration and are higher in disadvantaged populations and in subjects receiving suboptimal treatments, such as corticosteroid monotherapy or treatments without antimalarials (Barber & Clarke, 2017). This evidence can most likely be explained with higher accrual of chronic damage due to delayed diagnosis/treatment or inconstant disease monitoring leading to long-lasting uncontrolled disease activity. Nonetheless, even state-of-the-art care is insufficient to stably grant complete, drug-free remission to most patients with SLE. In fact, current diagnostic and treatment tools still lump patients with SLE into broad and non-specific categories and have a limited value in identifying clinically and prognostically relevant sub-phenotypes (Doria, Gatto et al., 2015). Developing translational models to describe SLE clinical variability along with biological correlates might therefore be crucial for the development of affordable prognostic markers and less toxic therapies. Antigen selectivity and coordination of long-term inflammatory responses constitute two intrinsic properties of T-lymphocytes and are increasingly exploited for the development of molecularly targeted diagnostic and therapeutic tools in multiple branches of human pathology (Milone, Xu et al., 2021, Oliveira, Ruggiero et al., 2015), while much less is known regarding the pathogenic role of T-cells and their potential medical applications in the setting of autoimmune/rheumatic diseases. Taking advantage of the nature of SLE

as a paradigm of immune dysfunction, this study aims to characterise antigen-specific T-cell responses in patients with SLE and correlate this evidence with clinical data.

Dynamics of T cell-responses in health and disease

Physiology

T lymphocytes lie at the apex of the adaptive immune response and coordinate the flow of antigen information from innate immune cellular and humoral sensors to effector T and B cells as well as back to innate effectors to promote threat recognition and neutralisation, besides maintaining tolerance to self structures. Two main subsets of T lymphocytes are defined based on their main biological function: CD4+ (helper) T cells and CD8+ (cytotoxic) T cells. Aberration in T cell function are associated with immunodeficiency, allergy and autoimmunity. Under physiological conditions, double positive (CD4+, CD8+) T cells residing in the thymus undergo a two-step process of selection and maturation into single positive (CD4+ or CD8+) naïve T cells, based on the affinity of their T cell receptor (TCR) for an array of self antigens presented through the major histocompatibility complex (MHC) repertoire of each individual. Failure of optimal negative and positive T cell selection in the thymus due to genetic defects such as those involving the AIRE (Autoimmune Regulator) gene are associated with multidirectional immune dysfunction with coexisting immunodeficiency and autoimmunity. After antigen encounter through antigen presenting cell presentation, naïve CD4+ and CD8+ cells further differentiate into increasingly committed T cell populations, which can in turn be classified based on stemness capacity and biological activity. Stem-cell memory T cells (T_{SCM}) are minimally differentiated antigen-experienced CD4+ or CD8+ T cells with the ability to repopulate a whole set of downstream antigen-specific T cell subpopulations (Gattinoni, Speiser et al., 2017). Monitoring antigen-specific T_{SCM} counts after haematopoietic stem cell transplantation is crucial for prognostic stratification in terms of variety and robustness of regained immunological competence (Cieri, Oliveira et al., 2015, Oliveira et al., 2015). Central memory T cells (T_{CM}) are supposed to constitute the step following T_{SCM} in terms of stemness. Within the CD4+ lineage, T_{CM} might constitute the memory reservoir of follicular helper and germinal centre follicular helper T cells (T_{FH} and $_{(GC)}T_{FH}$, respectively). Similarly, effector memory T cells (T_{EM}) lie downstream T_{CM} in the

differentiation cascade of T cells and constitute the memory counterpart of proper effectors. Effector cells (T_{EFF}) are in charge of the bulk production of cytokines characterising a given inflammatory event and expand following sustained stimulation with a trigger antigen (Ruterbusch, Pruner et al., 2020).

Polarisation within the CD4+ lineage might in turn vary considerably according to the nature of the inciting stimuli and constitutes an overlapping layer of complexity within T cell classification. Accordingly, distinct subpopulations of T_{FH}/T_{CM} and T_{EFF}/T_{EM} might arise from different inflammatory environments (Ruterbusch et al., 2020). A T helper 1 (Th1) environment arises under high-IL12 conditions and is characterised by enhanced production of interferon gamma (IFNγ), recruitment and activation of innate immunity and cellular responses towards viruses and intracellular pathogens. Conversely, a T helper 2 (Th2) environment, leading to the generation of T_{FH2} and T_{EFF2} subsets, typically promotes B-cell differentiation, eosinophil-mediated responses and tissue fibrosis following stimulation by parasites, venoms and other environmental antigens (Ruterbusch et al., 2020). A Th2 environment involves the release of IL4, IL5, IL13 and IL33. T helper 17 cells (Th17) differentiate in response to a cytokine milieu characterised by abundance of IL6, IL23 and transforming growth factor beta (TGFβ) and constitute a standalone third type of inflammatory environment (Sallusto, 2016). Th17 secrete an array of cytokines including the prototypical IL17A and IL17F, which in turn are responsible for neutrophil activation and potentiation of multiple cytokine release from epithelial, endothelial and innate immune cells (Acosta-Rodriguez, Rivino et al., 2007). Besides the three main branches of T helper cell differentiation (Th1, Th2, Th17) additional Th cell subtypes with peculiar functional specialisations have been progressively discovered, including plastic populations of Th1/Th2, Th1/Th17 and Th2/Th17 cells (Sallusto, 2016). IL9-secreting Th9 cells constitute a distinct population of Th cells within the Th2 biological spectrum, with potential non redundant function in the response to selected parasites. Similarly, Th22 cells arise under Th17-like conditions being however unable to release IL17 and may have a role in controlling pathogens affecting the skin. Non-classic Th1 cells or Th1-star (Th1*) cells constitute a recently defined subset within the spectrum of Th17 responses, showing a potential specialisation in contrasting infections due to intracellular pathogens (such as *Mycobacteria*) and a limited role in viral infections (Sallusto, 2016). Th1* cells are characterised by the

expression of both Th1-specific and Th17-specific surface markers such as CXCR3 and CCR6.

Regulatory T cells constitute an additional branch of the CD4+ T lymphocyte lineage and play a crucial role in immunosuppression and maintenance of tolerance. Regulatory cell subsets have also been described within the CD8+ T and B cell lineages (Grant, Liberal et al., 2015)

Allergy

T lymphocytes contribute to the promotion of hypersensitivity reactions both of immediate and delayed type. In the setting of atopy, molecular diagnostics has revolutionized the allergy practice and has disclosed novel opportunity for T cell studies exploiting protein antigens with known, selective immunogenicity. Inhalant or food allergen-specific CD4+ T cells are readily and selectively detectable in the blood of allergic subjects and show a Th2-skewed phenotype consistent with enhanced IgE responses (Archila, Jeong et al., 2015, Kwok, Roti et al., 2010, Macaubas, Wahlstrom et al., 2006). Furthermore, allergen-specific T cell phenotype varies with immunomodulatory therapies (Wambre, DeLong et al., 2012). In contrast to atopy, the molecular bases of immediate- and delayed-type hypersensitivity reactions to drugs have less been defined (Adam, Pichler et al., 2011). In the setting of penicillin allergy, some authors employed penicilloylated peptides to induce and measure T cell responses (Azoury, Fili et al., 2018, Nhim, Delluc et al., 2013, Padovan, Bauer et al., 1997). Penicilloylated peptides have been shown to bind effectively to several HLA-DRB1 molecules and to elicit detectable IFNγ responses as measured by ELISpot assays (Azoury et al., 2018, Nhim et al., 2013). Interestingly, effective anti-penicillin responses have consistently been detected in cells from healthy donors, possibly suggesting that potential sensitisation to beta-lactams is common in the general population with tolerance being mostly due to regulatory mechanisms (Azoury et al., 2018). No study has so far employed direct *ex vivo* techniques for T cell visualisation such as MHC multimers to characterise anti-beta lactam responses in allergic individuals, preventing dissection of non-specific or artificially skewed T cell responses due to cell stimulation protocols from natural mechanisms of allergic inflammation.

Autoimmune diseases

Systemic vasculitides

Inflammation of the large, medium and small blood vessels occurs in a multitude of conditions including infectious and autoimmune diseases. In this latter setting, systemic vasculitides can develop as a complication of other rheumatologic disorders or as primary immune-mediated diseases. The clinical-pathophysiological nomenclature by the Chapel Hill Consensus Conference identifies four major disease groups (small, medium, large and variable vessel vasculitides), besides three other special categories (single-organ vasculitides, vasculitides associated with probable aetiology and vasculitides associated with systemic diseases) (Jennette, Falk et al., 2013).

Extensive evidence from clinical observations and animal models supports a view of large vessel vasculitides as T cell-dependent diseases (Brack, Geisler et al., 1997, Weyand, Schonberger et al., 1994). T cells orchestrate the downstream inflammatory and hyperplastic response causing disruption of the vessel wall architecture and the subsequent formation of vessel stenosis and/or aneurysms. In giant cell arteritis (GCA), the inflammatory response is centripetal: T-cell infiltration of the vessel wall occurs through the adventitial vasa vasorum and is modulated by the local inflammatory niche, including vessel residing dendritic cells (Wen, Shen et al., 2017). Activation of CD4+ T cells prevails in GCA and is more prominently skewed towards a Th1/Th17 phenotype, consistent with the granulomatous nature of the disease (Brack et al., 1997, Deng, Younge et al., 2010). Th17-biased responses in GCA appear to account for systemic symptoms and vessel wall remodelling due to a direct correlation with local matrix metalloprotease expression. Th17 cells are also more susceptible to corticosteroid treatments which constitute the mainstay of therapy in this disorder. Th1-driven responses are instead more resistant and might account for glucocorticoid-refractory cases (Deng et al., 2010). Takayasu's arteritis (TAK) is a large vessel vasculitis with distinct clinical and pathophysiological features compared to GCA. From a clinical point of view TAK affects young women (in contrast to the elderly-skewed demographics of GCA) causing occlusion or aneurysmatic deformation of the upper and lower aorta and its major branches including coronary arteries. Similar to GCA, TAK is associated with selected HLA profiles (Carmona, Coit et al., 2017, Dong, Kimura et al., 1992, Lv, Wang et al., 2015) and with enhanced Th1 and Th17 responses.

Genetically determined and acquired alterations of T cell function constitute a characteristic feature of patients with small-vessel vasculitides such anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV). Specifically, patients with AAV frequently bear polymorphisms in key genes for T cell activation and co-stimulation control such as protein tyrosine phosphatase N22 (PTPN22) and cytotoxic T lymphocyte antigen 4 (Alberici, Martorana et al., 2014), besides selected HLA variants. Patients with AAV are also characterised by enhanced Th1/Th17 responses, which might be selectively triggered by exposure of key autoantigens such as proteinase 3 (Abdulahad, Stegeman et al., 2008, Kallenberg, 2011). A recent study also identified a shared B and T cell myeloperoxidase epitope and, by the use of MHC multimer staining techniques, identified circulating anti-myeloperoxidase-specific CD4+ T cells with a Th17 phenotype in patients with AAV (Free, Stember et al., 2019). Consistent with similar evidence in other disease settings, circulating T_{EFF}/T_{EM} cells decrease during active disease, reflecting their re-localisation into target tissues. Patients with AAV and active nephritis are in fact characterised by enhanced urinary concentration of T_{EM} cells in contrast to the circulating blood (Abdulahad, Kallenberg et al., 2009). Deficit of T regulation have also been advocated to contribute to the pathogenesis of AAV (Abdulahad, Stegeman et al., 2007).

Rheumatoid arthritis

Rheumatoid arthritis is a frequent autoimmune disease characterised by prominent symmetric involvement of small joints and potential extra-articular complications. Although clearly distinct in terms of demographics, clinical presentation and general pathophysiology, rheumatoid arthritis and GCA share a common HLA-DR risk allele Similar to GCA, rheumatoid arthritis is characterised by a crucial role of Th17 cells for progression and maintenance of the inflammatory response. Specifically, Th17 cells promote TNFα-driven joint inflammation and B cell activation. Persisting inflammation leads to tissue damage with enhanced exposure of aberrantly edited self antigens, such as citrullinated peptides. This in turn, leads to the development of the hallmark anti-citrullinated peptides and chronicisation of the inflammatory status. Anti-citrullinated peptide T cell immunity plays a crucial role in initiating this process. Consistently, HLA-DRB1*04:01-restricted citrullinated peptides bound to MHC multimers allowed direct *ex vivo* visualisation of antigen- and disease-specific CD4+ T cells from the circulating

blood (James, Rieck et al., 2014), synovial fluid and tissue of patients with rheumatoid arthritis (Pieper, Dubnovitsky et al., 2018). Interestingly, arthritogenic antigen-specific CD4+ T cells from patients with rheumatoid arthritis are characterised by a memory phenotype with prominent expansion of the T_{SCM} compartment and respond to tumour necrosis factor alpha (TNFα) modulation mirroring the clinical phenotype (Cianciotti, Ruggiero et al., 2019, James et al., 2014, Pieper et al., 2018).

Systemic lupus erythematosus

Epidemiology

Multiple studies attempted to estimate SLE prevalence in the general population. A 2006 metanalysis by Danchenko and colleagues suggests that SLE affects up to 159 persons for every 100,000 inhabitants, with significant regional and ethnical variations (Danchenko et al., 2006). More recent data indicate that this figure might be underestimated (Cortes Verdu, Pego-Reigosa et al., 2020, Pablos, Abasolo et al., 2020). SLE prevalence in Italy is supposed to range from 51 to 71 cases per 100,000 inhabitants according to at least three studies (AMRER (Associazione Malati Reumatici Emilia-Romagna), 2015, Benucci, Del Rosso et al., 2005, Govoni, Castellino et al., 2006). These figures are roughly higher than the average expected prevalence of SLE in Europe (Danchenko et al., 2006).

Women with SLE are nine times more frequent than men, consistent with the inflammatory effect of oestrogens and with the role of X-inactivation escape in enhancing innate immune responses (see below). Ultraviolet light, viral infections, low vitamin D, selected drugs and polymorphisms in a multitude of genetic loci constitute additional risk factors for the development of SLE (Simard & Costenbader, 2015). The peak incidence of the disease occurs during the III decade of life, although juvenile or late-onset cases may also develop, usually with less evidence of disproportion among female and male patients. Early-onset disease is usually more aggressive and shows a relatively higher prevalence of renal and neuropsychiatric involvement (Ambrose, Morgan et al., 2016, Artim-Esen, Sahin et al., 2017, Ramirez, Tejera-Segura et al., 2018). Furthermore, the first months following overt disease onset are usually characterised by a more aggressive course, while a relatively low frequency of disease flares is observed in longstanding disease (Gerosa, Ramirez et al., 2020, Holmqvist, Simard et al., 2015, Lim, Pullenayegum

et al., 2018, Nossent, Cikes et al., 2007, Piga, Floris et al., 2017, Scalzi, Hollenbeak et al., 2010). As anticipated, SLE can have a major impact on patients' quality of life, and social and economic balance. On the other hand, social and economic disparities have a role in SLE prognosis and might be more significant than genetics and disease-related variables in affecting the course of the disease (Joseph, Prasad et al., 2021, Ugarte-Gil, Pons-Estel et al., 2016). Long-term disability and mortality related to SLE have significantly decreased over time thanks to the introduction of immunosuppressive treatments, improved and more diffuse awareness of the disease in the general population and medical community and adoption of personalised approaches for patient care (Banchereau, Hong et al., 2016, Lever, Alves et al., 2020, Nossent et al., 2007). Despite the fall of acute inflammatory events as causes of SLE-related mortality, chronic complications due to prolonged smouldering activity, progressive damage accrual and drug-related effects, such as cardiovascular diseases and metabolic disorders still represent major mortality-related factors for patients with SLE. These considerations further support the need for novel tools to dissect the pathophysiological events deploying in each individual patient to early intercept potential drivers of long-term complications.

Clinical features

Inflammatory manifestations

The spectrum of clinical manifestations attributable to SLE is very broad and might vary significantly among patients and in the same subject during the course of the disease, according to the modulatory effect of the environment and of treatments (Agmon-Levin, Mosca et al., 2012, Banchereau et al., 2016).

Cutaneous and musculoskeletal manifestations constitute the most frequent features of SLE and might present both as a definite clinical cluster (Terao, Yamada et al., 2014) or as part of more complex combination of symptoms. Skin manifestations are reported in up to 85% of patients and the word lupus itself is a medieval medical term standing for face skin inflammation. In fact, photosensitivity is a hallmark of the disease and is particularly frequent in most exposed skin regions, such as face, upper trunk and limbs. Malar rash, classically described to spare the light-protected rhino-labial region, constitute the most typical manifestation of photosensitivity. According to the clinical-pathological Düsseldorf classification (Kuhn & Landmann, 2014, Sontheimer, 2004),

acute rash due to ultraviolet light-induced inflammation is usually opposed to subacute (SCLE) and chronic (CCLE) cutaneous lupus. Subacute lesions usually present as erythematous papules with a polycyclic appearance and also tend to occur more frequently in photosensitive areas. Discoid lupus, consisting of violaceous to brownish infiltrated plaques evolving into fibrotic and discoloured lesions, constitute the most frequent manifestations of CCLE. Additional CCLE lesions include mucous manifestations (oral and nasal ulcers), lupus panniculitis (also known as lupus profundus) and chilblains lupus. This latter sign is part of a broader spectrum of manifestations due to small vessel dysfunction and/or damage, encompassing Raynaud's phenomenon and cutaneous vasculitis. Alopecia is another frequent and invalidating skin manifestation of SLE. Cutaneous lupus (especially SCLE) is usually associated with positive anti-SSA(Ro)/SSB(La) antibodies (aSSA, aSSB respectively) and has a particularly strong association with HLA-DRB1*03 (Diaz-Gallo, Oke et al., 2021). Histologically it is characterised by prominent inflammation of the epidermal/dermal border (interface dermatosis) with variable degrees of lymphocyte infiltration and deposition of immunoglobulins and/or complement (evident as the so-called lupus band on immunofluorescence assays). The likelihood of developing scars and chronic skin damage is higher in patients with CCLE than in patients with SCLE and ACLE. Conversely, association with extracutaneous manifestations is more frequent in acute forms.

Joint involvement is also frequent in patients with SLE, especially in late-onset subsets (Ambrose et al., 2016), with an estimated prevalence of up to 90% (Petri, 2007). Non-erosive synovitis or tenosynovitis is usually regarded as the typical pattern of joint disease in SLE, as opposed to the erosive pattern of rheumatoid arthritis. However, overlap syndromes encompassing seropositive rheumatoid arthritis and SLE (rhupus) might also occur. In addition, erosive features are increasingly recognised in the absence of rheumatoid serology (namely anti-citrullinated antibodies and/or rheumatoid factor) and even in patients with Jaccoud's arthropathy, a reducible joint deformity of ill-defined pathophysiology, usually regarded as SLE-specific (Di Matteo, Smerilli et al., 2021). Novel evidence also challenges the paradigm of SLE selectivity for the synovial tissue, suggesting that enthesitis might further expand the heterogeneous spectrum of SLE phenotype and correlate with general disease activity (Di Matteo, Filippucci et al., 2018).

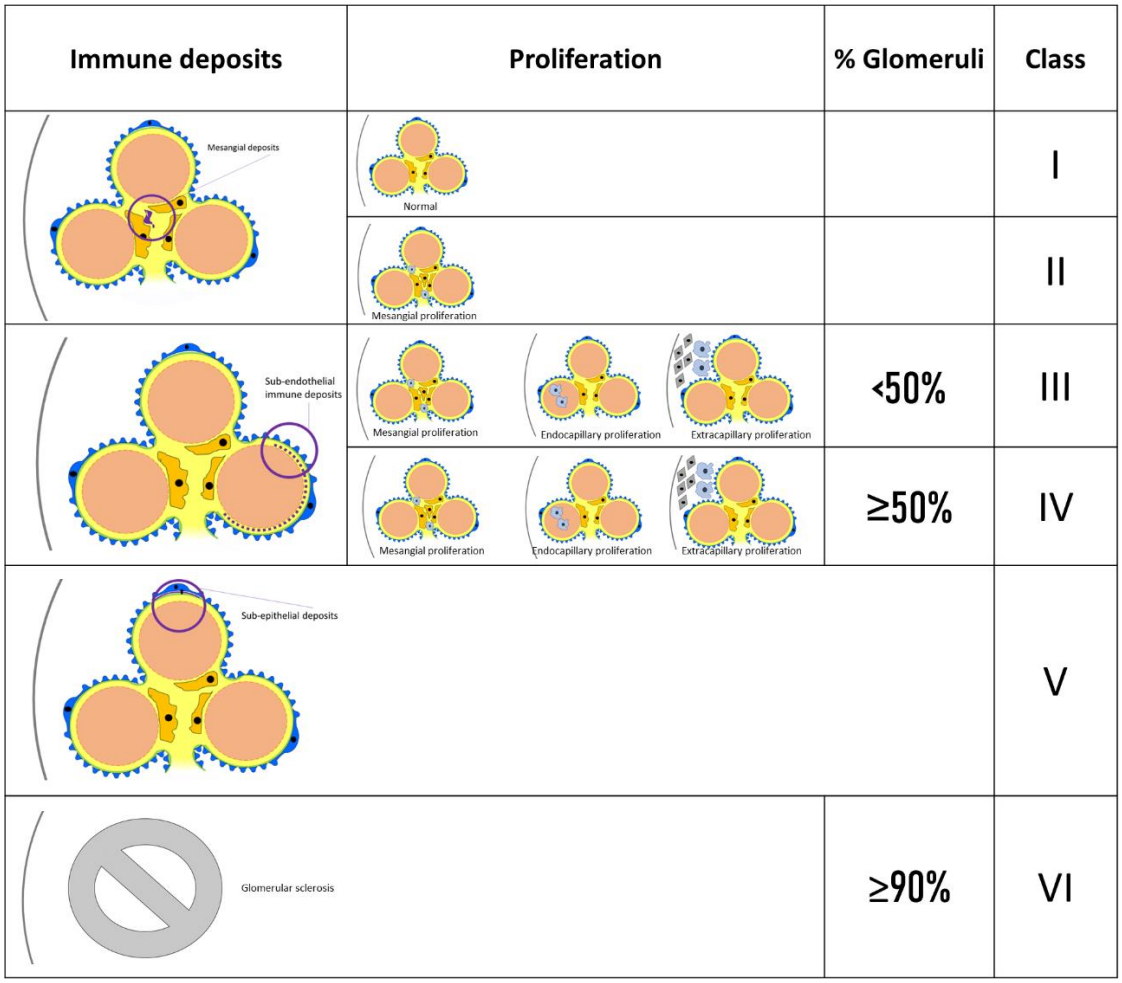
Data from multiple studies also indicate that subclinical joint inflammation might be particularly frequent, leading to chronic damage despite clinical monitoring, in the absence of serial imaging (Piga, Gabba et al., 2016, Tani, Carli et al., 2018).

Constitutional symptoms constitute a frequent but elusive aspect of SLE clinical spectrum. Fatigue is the commonest symptom in this category, as it may affect more than 80% of patients. Its clinical course is often uncoupled from general disease activity and might be related to multiple mechanisms including systemic inflammation, abnormal iron metabolism or alterations in brain connectivity and emotional input processing (Cleanthous, Tyagi et al., 2012, Harboe, Greve et al., 2008, Moroni, Mazzetti et al., 2021, Wincup, Sawford et al., 2021, Wiseman, Bastin et al., 2017). Enhanced activation of the reticuloendothelial system is also common, especially in patients of younger age and can manifest as low-grade to high fever and diffuse lymph-node enlargement. These manifestations can further evolve or be complicated by the development of haemophagocytic lymphohistiocytosis or Kikuchi-Fujimoto's disease. Distinguishing florid inflammation from haematological malignancies is often challenging both at a clinical and histological level (Gavand, Serio et al., 2017, Henter, Horne et al., 2007, Kim, Kwok et al., 2012, Petri, Kawata et al., 2013).

Lupus nephritis constitutes the main cause of SLE-related morbidity and affects about 40% of patients during their disease course. A histopathological classification identifies six patterns of glomerular involvement in terms of immune deposits and/or proliferation (**Figure 1**). Class I and II glomerulonephritis correspond to the presence of minimal mesangial deposits (I) and/or proliferation (II), with no or isolated mild urinary sediment abnormalities as clinical correlates. Class III and IV are characterised by sub-endothelial deposits and hypercellularity at the level of either mesangial, endocapillary, and/or extracapillary spaces due to proliferation of resident cells and/or infiltration by circulating leukocytes. Class IV affects 50% or more of the glomeruli, in contrast to class III, and is further sub-classified into segmental (class IV-S) or global (class IV-G) forms in case of lesions in less than 50% vs 50% or more of each glomerular tuft. Class III and IV are also classified according to the presence of active and/or chronic lesions. Class III and IV constitute the most frequent and aggressive forms of lupus nephritis and usually present with nephritic syndromes with abnormal proteinuria, active urinary sediment, with or without hypertension and reduced filtration rates. Class V glomerulonephritis refers to

membranous nephropathy with or without coexistent proliferation. This form usually presents with nephrotic manifestations, is less aggressive and rapidly evolving than class III and IV and shows an overall better prognosis despite being less affected by immunosuppression (Farinha, Pepper et al., 2020). Class VI corresponds to irreversible renal failure with loss of functional glomeruli and diffuse sclerosis.

Figure 1: histopathological classification of glomerular involvement in SLE.



This figure depicts a simplified version of the "International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification of lupus nephritis" (Weening, D'Agati et al., 2004).

Additional criteria have been set to quantitate the degree of activity and chronic damage. Besides glomerular lesions, tubular involvement can also develop in patients with SLE and contribute to renal impairment. Furthermore, vascular lesions secondary to ischaemia or primary vessel inflammation can also occur (Bajema, Wilhelmus et al., 2018, Strufaldi, Menezes Neves et al., 2021).

Neuropsychiatric manifestations of SLE (NPSLE) constitute some of the most challenging aspects of the disease. In fact, the spectrum of NPSLE encompasses a variety of acute and chronic conditions affecting either the central, peripheral of autonomic nervous system through micro- or macrovascular inflammatory and/or ischaemic mechanisms, humoral and cell-mediated autoimmunity and alterations in neuronal connectivity (Bonacchi, Rocca et al., 2020, Govoni, Bortoluzzi et al., 2016, Preziosa, Rocca et al., 2019, Ramirez, Canti et al., 2019a, Ramirez, Lanzani et al., 2015a, Unterman, Nolte et al., 2011). Stroke, epilepsy and psychosis are among the most frequent manifestations of SLE at a central neurological level. Headache and mood disorders are also frequent but less specific, due to their high prevalence in the general population (Ainiala, Loukkola et al., 2001, Hanly, 2014). Peripheral neuropathy is also not uncommon in patients with SLE and is therefore listed among the items of the systemic lupus erythematosus international collaborating clinics (SLICC)/American College of Rheumatology (ACR) damage index (SDI) besides the ACR 19-item nomenclature of NPSLE features (ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature, 1999). Attribution of a neuropsychiatric event to SLE is a challenging task due to the lack of univocal tests for the diagnosis and the absence of specific imaging features (Govoni et al., 2016, Jeong, Her et al., 2015, Sarbu, Alobeidi et al., 2015, Sarbu, Toledano et al., 2017, Sibbitt, Brooks et al., 2010). Nonetheless, attribution algorithms based on temporal criteria and on the presence/absence of favouring and confounding factors have recently been developed and validated (Bortoluzzi, Fanouriakis et al., 2017, Bortoluzzi, Scire et al., 2015).

Virtually all internal organs can be involved in the pathogenic processes of SLE. Cardiopulmonary manifestations are more often limited to inflammation of the serosal layers (pleuritis and pericarditis). Nonetheless, autoimmune myocarditis, parenchymal and interstitial lung involvement are also part of the spectrum of SLE and might have a significant impact on morbidity and mortality (Pego-Reigosa, Medeiros et al., 2009, Tanwani, Tselios et al., 2018). Aseptic endocarditis, generally in association with anti-phospholipid antibodies (aPL) might also be more frequent than expected in patients with SLE (Vivero, Gonzalez-Echavarri et al., 2016).

Liver involvement is virtually indistinguishable from isolated autoimmune hepatitis. Pancreatic involvement is uncommon in SLE but may occur either as an acute sterile

inflammatory event, chronically in the context of overlapping Sjögren's syndrome or as the side effect of drugs. Gastrointestinal manifestations are also infrequent (possibly also due to underdiagnosis) and ill-defined and might encompass serosal inflammation (sterile peritonitis), visceral vasculitis or, less frequently, primary enteritis. The course of gastrointestinal involvement might be poorly related to that of the disease in general (Li, Xu et al., 2017, Maruyama, Nagashima et al., 2018).

Altered haematopoiesis is a distinctive feature of SLE and can involve either red blood cells, leukocytes and platelets through different mechanisms. Micro/normocytic inflammatory anaemia is very frequent in SLE and often overlaps with iron deficiency or imbalanced iron metabolism. Coombs-positive haemolytic anaemia is more specific of SLE and often coexists with immune-mediated thrombocytopenia. This clinical cluster, usually referred to as Fisher-Evans' syndrome can also precede the onset of SLE by several years. Leukopenia is another hallmark feature of SLE and other interferon-driven conditions and can be due to either enhanced leukocyte migration into target tissues or to impaired bone marrow responses. Lymphopenia is more often observed although low neutrophil counts can also be found in up to one third of patients. Both conditions are usually benign and show no direct correlation with increased infection rates. Therefore, they do often not require specific treatments (Carli, Tani et al., 2015).

Dysfunctional humoral adaptive response is crucial in the pathogenesis of SLE. Accordingly, a multitude of autoantibodies can be detected in patients with this disease and variably contribute to the clinical phenotype. The following section will briefly present most significant antibodies for clinical practice. Additional pathophysiological information will be provided from page 39. Antinuclear antibodies (ANA) are almost invariably detected in the setting of SLE although are also commonly seen in patients with other connective tissue diseases, patients with thyroid immune-mediated disorders and healthy subjects. Anti-double stranded DNA (ADNA) antibodies are more specifically associated with SLE and can be detectable in up to 98% of patients (Conti, Ceccarelli et al., 2015). Anti-nucleosome and anti-histone antibodies overlap with or extend the diagnostic spectrum of ADNA but are not routinely employed in clinical practice (Ghiggeri, D'Alessandro et al., 2019). Anti-Smith (aSm) antibodies are specific but significantly less prevalent. Additional frequent anti-extractable nuclear antigens antibodies include aSSA and aSSB antibodies (see above) and anti-ribonucleoprotein

(aRNP) antibodies. Antiphospholipid antibodies (aPL) identify a standalone serological-pathophysiological subset within SLE and can in fact develop (with or without clinical correlates) also in patients with other rheumatic disorders or in otherwise healthy subjects. Anticardiolipin (aCL) and anti-beta 2 glycoprotein I (aB2GPI) antibody assays along with lupus anticoagulant test constitute the most robust and widely accepted tests for aPL detection, while antibodies against additional targets are progressively being discovered and validated for clinical use (Chighizola, Raschi et al., 2015, Gaspar, Cohen et al., 2020). Up to 40% of patients with SLE are aPL carriers, while up to 15% also show clinical signs of thrombosis/ischaemia and/or pregnancy morbidity consistent with anti-phospholipid syndrome (APS) (Cervera, Serrano et al., 2015, Ramirez, Efthymiou et al., 2019c).

Immunodeficiency

General considerations

Infectious agents constitute the archetype stimulus accounting for the evolution of the immune system. Accordingly, pathogen-related triggering of self-sustained inflammatory/autoimmune phenomena have repeatedly been described in a multitude of clinical and preclinical settings (Cui, Zhou et al., 2021, Jacobs, Giovannoni et al., 2020, Jasemi, Erre et al., 2021, Kain, Exner et al., 2008, Lloyd, Tamhankar et al., 2021). Re-activation of endogenous retroviral elements might also contribute to inflammatory disease onset or flaring (Khadjinova, Wang et al., 2021, Wang, Hefton et al., 2021). Nonetheless, the actual clinical and pathological relevance of infectious and inflammatory events taken singularly remains elusive. While an association between immunodeficiency and autoimmune manifestations is well established in congenital disorders of the immune response (Azizi, Ghanavatinejad et al., 2016, International Union of Immunological Societies Expert Committee on Primary, Notarangelo et al., 2009), less is known about the pathophysiological role of infections in patients with multi factorial immune-mediated diseases such as SLE (Sawada, Fujimori et al., 2019).

Patients with SLE have a more than two-fold increased risk of infections such as pneumonia, herpes zoster and tuberculosis and a three-fold increased risk of severe infections compared to the general population (Pego-Reigosa, Nicholson et al., 2021). Accordingly, infections constitute a leading cause of hospitalisation and mortality in patients with SLE (Goldblatt, Chambers et al., 2009, Navarro-Zarza, Alvarez-Hernandez

et al., 2010). Regarding the site of infection, the respiratory and urinary tracts are more frequently involved, although skin and soft tissue infections are also highly prevalent (Danza & Ruiz-Irastorza, 2013). Patients with more severe disease in terms of extension (number of involved tissues and organs) and activity, and/or with a higher burden of immunosuppression bear a higher risk of infection (Bosch, Guilabert et al., 2006, Duffy, Duffy et al., 1991, Rua-Figueroa, Lopez-Longo et al., 2017, Ruiz-Irastorza, Olivares et al., 2009, Zonana-Nacach, Camargo-Coronel et al., 2001). Notably, however, effective immunomodulation with the use of antimalarials has a protective effect towards infections (Ruiz-Irastorza et al., 2009). More specifically, antimalarials neutralise the increased infection risk attributable to (moderate to low dose) corticosteroids according to epidemiological evidence (Herrinton, Liu et al., 2016). Annual influenza infection is a major trigger of SLE flares, in contrast to anti-influenza vaccination (Chang, Chang et al., 2016, Joo, Kim et al., 2021, Touma, Gladman et al., 2013). In addition, new-onset infection or reactivation of multiple pathogens have also been associated to SLE activity.

Herpesviruses

Vulnerability to the reactivation of herpesviruses, especially Epstein-Barr virus (EBV) and varicella-zoster virus (VZV) is a clinical/pathophysiological hallmark of SLE (Chakravarty, Michaud et al., 2013). Cytomegalovirus (CMV) infection also has a disproportionately high prevalence among patients with SLE, although the role of CMV reactivation in triggering lupus flares appears less relevant compared to EBV (Draborg, Rasmussen et al., 2018, Rider, Ollier et al., 1997). Multiple pathogenic factors might account to herpesvirus infection/reactivation susceptibility in patients with SLE. Dysfunctional interferon responses might be particularly detrimental for the control of viral infections (see also below regarding the current coronavirus pandemic)(Gupta, Nakabo et al., 2021a). Consistently, non-selective inhibition of interferon alpha (IFNα) prompts an increase in herpes zoster rates in patients with SLE (Khamashta, Merrill et al., 2016, Tummala, Abreu et al., 2021). In addition, exhaustion of T-cell responses due to persistent low-grade viral replication during chronic infection has been hypothesised as a key factor in dysregulated anti-herpetic responses, independent of treatment status (Draborg, Jacobsen et al., 2014).

Similar to other autoimmune disorders such as multiple sclerosis (Jacobs et al., 2020), more consistent evidence exists about a potential role of EBV in the pathogenesis of SLE. This consideration is coherent with the high prevalence of EBV infection in the general population. Specifically, seroprevalence of previous EBV infection in Italy exceeds 70-90% (Leogrande & Jirillo, 1993, Pordeus, Barzilai et al., 2008, Shapira, Poratkatz et al., 2012). Patients with SLE have normal or almost normal humoral immune responses to EBV, but fail to develop efficient cytotoxic T-cell responses and show higher number of viral copies in the circulating blood independent of treatment (Kang, Quan et al., 2004, Moon, Park et al., 2004, Tsokos, Magrath et al., 1983). Anti-EBV T cell responses have been shown to correlate inversely with disease activity (Draborg et al., 2014). Less is known about the potential features of CD4+ T-cell responses, which might behave independently on disease activity (Kang et al., 2004).

COVID-19 and anti-SARS-CoV-2 vaccines

During the last two years, a novel systemic infectious disease has spread pandemically across the World causing immeasurable costs in terms of human lives, acute and chronic morbidity, social relations, political and economic stability. This disease has been named COVID-19 after its causative agent, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which was isolated in late 2019. Briefly, COVID-19 is characterised by prominent involvement of the upper and lower respiratory tract and can be complicated by severe systemic inflammatory manifestations, thrombotic/ischaemic events as well as neurological and endocrine sequelae. Morbidity and mortality due to multi-organ failure are unfortunately frequent in the absence of pre-emptive immunisation or adequate life support (Ciceri, Beretta et al., 2020, Guan, Ni et al., 2020, Rovere Querini, De Lorenzo et al., 2020).

As a result of COVID-19 becoming an unavoidable priority, the whole scientific community has "forcibly" been converted to study the multifaceted aspects of the disease, of its sequelae and of therapeutic and prophylactic strategies aiming at its containment. By doing so, scientists also seized an occasion to reconsider and update consolidated knowledge in General/Internal Medicine and other subspecialties, taking advantage of readily available data on wide cohorts of patients. Along with this line, COVID-19 pandemic also became an unprecedented occasion to study the interaction of immune-

mediated diseases such as SLE and infection and the potential impact of vaccination practices in patients with immune dysfunction. Initial hypotheses suggested that patients with autoimmune diseases and specifically with SLE might indeed have multiple factors conferring theoretical protection from the most severe forms of COVID-19. In fact, while advanced age and male sex are known risk factors for COVID-19-related morbidity and mortality, SLE incidence is higher among young women. Furthermore, defective interferon response is a hallmark of coronavirus infections and has a prominent role in the pathophysiology of COVID-19, while higher levels of systemic interferon levels are detected in patients with SLE (BastardRosen et al., 2020, Garcia-Romo, Caielli et al., 2011).

Indeed, patients with SLE and other systemic immune-mediated disorders have a significant, though modest, increase in their individual risk of infection and complicated COVID-19 course (Ramirez, Gerosa et al., 2020, Ramirez, Moroni et al., 2020, Scire, Carrara et al., 2020, Strangfeld, Schafer et al., 2021). From a pathogenic point of view, these data are consistent with the contention that interferon responses in SLE are more properly dysregulated rather than simply inflated (Banchereau et al., 2016, Gupta et al., 2021a, Gupta, Nakabo et al., 2021b, Sawalha, Zhao et al., 2020). Accordingly, anti-interferon antibodies are detectable both in patients with SLE and with dysfunctional response to COVID-19 (Bastard et al., 2020, Gupta et al., 2021a, Gupta et al., 2021b). Clinically, this evidence is mirrored by the association between active disease and severe COVID-19, independent on treatments (Strangfeld et al., 2021). While there is no clear evidence of an association between activity in specific organ/tissue domains and COVID-19, patients with comorbid asthma might be particularly at risk, consistent with a model of generalised immune dysfunction as a hallmark of severe SLE (Ramirez, Argolini et al., 2021). Furthermore, while the protective effects of young age are likely lost in long-surviving patients with SLE, disease-related alterations in tissue susceptibility to SARS-CoV-2 entry might favour complicated COVID-19 courses (Sawalha et al., 2020).

Vaccination against SARS-CoV-2 has now consistently been introduced into clinical practice, with dramatic changes in infection and death rates at a population level. mRNA-based vaccines showed the best efficacy and safety performances towards COVID-19, at least in part due to their excellent immunogenicity profile and ability to stimulate a robust innate response (Pardi, Hogan et al., 2018, Polack, Thomas et al.,

2020). This feature, however, constituted a potential matter of challenge for patients with immune-mediated diseases, including SLE (Ramirez, Asperti et al., 2021). In fact, both nucleic acids and their liposomal covers might trigger Toll-like receptor (TLR)-related pathways and downstream inflammatory responses, mimicking disease-related pathogenic events (Pardi et al., 2018). Although, episodic, mostly self-limited cases of vaccine-associated flares have been reported (Barbhaiya, Levine et al., 2021), anti-SARS-CoV-2 mRNA vaccines have generally been tolerated by patients with autoimmune diseases, including SLE. Yet, adverse events were more frequent among patients with coexistent allergy history (Nittner-Marszalska, Rosiek-Biegus et al., 2021, Ramirez, Della-Torre et al., 2021), suggesting that impaired tolerance to innocent antigens might associate with hypersensitivity and autoimmune manifestations.

Allergic manifestations

Associations among autoimmune and allergic manifestations in SLE are far less defined than those linking infections to disease flares, possibly due to the contention that immunosuppression could prevent allergen sensitisation and hypersensitivity reaction occurrence. This concept, however, is not supported by clinical evidence even in settings with need for more profound immunosuppression than SLE, such as organ transplant recipients (Dehlink, Gruber et al., 2006, Guo, Fang et al., 2019). In addition, extensive methodological discrepancies exist among different studies. Three small-sized studies from the United Kingdom, Japan and Poland, suggest that allergy prevalence in SLE might be comparable or even lower to that of the general reference population, at least in terms of atopy and "classical" IgE-mediated mechanisms (Morton, Palmer et al., 1998, Sekigawa, Yoshiike et al., 2002, Wozniacka, Sysa-Jedrzejowska et al., 2003). This evidence is in contrast with other small studies (Diumenjo, Lisanti et al., 1985, Shahar & Lorber, 1997). Two larger population studies from Taiwan suggest that accrual of atopic manifestations additively increases the risk of SLE over time (Hsiao, Tsai et al., 2014, Shen, Tu et al., 2014). More consistent evidence links drug allergy and SLE (Parks, Biagini et al., 2010, Petri & Allbritton, 1992, Sequeira, Cesic et al., 1993, Wozniacka et al., 2003), although the hypothesis of a stronger allergy risk in SLE than in other rheumatic disorders is still controversial (Aceves-Avila & Benites-Godinez, 2008, Pope, Jerome et al., 2003). Antibiotic allergy has been suggested to account for the majority of

drug allergy manifestations in patients with SLE, despite the frequent use of drugs with potential allergenicity such as non-steroidal antinflammatory drugs (Petri & Allbritton, 1992, Pope et al., 2003). In this specific settings, sulphonamides have emerged as preferential culprit drugs (Petri & Allbritton, 1992, Pope et al., 2003). Besides hypersensitivity reactions, patients with SLE are also at increased risk of developing immediate-type hypersensitivity-like reactions such as urticaria and angioedema (Ferriani, Silva et al., 2015, Kolkhir, Pogorelov et al., 2016, Luo, Fan et al., 2019).

Diagnostics and treatment

Diagnostics

The diagnosis of SLE is currently based on a combination of clinical signs and laboratory features. For purposes of classification and inclusion in clinical and translational studies, multiple sets of criteria have been developed over time. The most widely used classification criteria are the revised 1997 ACR criteria and the 2012 SLICC criteria (Hochberg, 1997, Petri, Orbai et al., 2012). More recently, a joint effort by the EULAR and the ACR has led to the development of a novel classification algorithm with improved sensitivity and specificity compared to the previous criteria (Aringer, Costenbader et al., 2019). Dedicated algorithms have also been developed for the classification of NPSLE, in the absence of established and univocal laboratory or imaging tools for a definite diagnosis (ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature, 1999, Bortoluzzi et al., 2017).

Multiple tools have also been developed to measure damage accrual and disease activity in SLE. Accumulation of disability due to tissue and organ dysfunction following irreversible disease-related injury or drug-related toxicity is usually measured with the SDI (Gladman, Goldsmith et al., 2000). The SDI tool is an additive scoring system based on 46 items developed on a limited number of paradigmatic cases by a panel of experts and eventually validated in clinical practice. Increasing SDI scores are in fact correlated with patient prognosis in terms of both overall survival and quality of life (Hanly, 1997, Nossent, 1998, Rahman, Gladman et al., 2001, Stoll, Seifert et al., 1996).

There is much less consensus on the reliability of tools assessing disease activity, especially for purposes of evaluation of the effect of treatments. The SLE disease activity index (SLEDAI) is one of the simplest and most widely used scoring systems to quantitate

disease activity in SLE at a given timepoint. It has been developed through an iterative procedure of selection and eventual weighting of relevant features and takes into account both serological activity (low complement, positive aDNA) and clinical features (Bombardier, Gladman et al., 1992). With time, it has undergone multiple revisions and adaptations to respond to a more extended panel of clinical and research questions. Currently, the Safety of Estrogens in Lupus Erythematosus National Assessment SLEDAI (SELENA-SLEDAI) and the 30- or 10-day SLEDAI-2000 (SLEDAI-2K) version of the original SLEDAI score are most frequently employed in clinical practice and for research studies (Gladman, 2015, Gladman, Ibanez et al., 2002). The SLEDAI-2KG is an additional variant of the SLEDAI-2K accounting for the ongoing glucocorticoid therapy and possibly showing better performances in intercepting treatment efficacy (Touma, Gladman et al., 2018). Further variants of the score have also been developed to emphasize clinical over serological activity (clinical SLEDAI, cSLEDAI) or to conform to specific geographical settings (for example the Mexican version of the SLEDAI or MEX-SLEDAI) (Castrejon, Tani et al., 2014). A second branch of the SLEDAI "family" of disease activity scores encompasses tools able to integrate and measure disease activity variations over time. This set includes the adjusted mean SLEDAI, the SLEDAI-2K responder index 50 (SRI-50), the SLE flare index, and the more recent SLE disease activity score (SLEDAS) (Ibanez, Urowitz et al., 2003, Jesus, Matos et al., 2019). The European Consensus Lupus Activity Measurement (ECLAM) score is a compact disease activity score similar to the SLEDAI-2K score but with enhanced emphasis on laboratory features such as lymphopenia, complement variations over time, non-haemolytic anaemia and erythrocyte sedimentation rate (Mosca, Bencivelli et al., 2000). The British Isles Lupus Assessment Group (BILAG) score has a radically diverging design as it is based on a composite array of mixed qualitative and quantitative scores addressing disease activity by systems or "domains". Specifically, the latest version of the score (BILAG-2004) encompasses nine domains (constitutional, mucocutaneous, musculoskeletal, neurological, cardiorespiratory, ophthalmic, gastrointestinal, renal, haematological). Each domain can be assigned a qualitative score from A (severe activity) to D-E (no activity in a previously involved or uninvolved domain, respectively) based on the expected Physician's intention-to treat. Each qualitative score is generated based on an algorithm taking into account the presence of

one or more active clinical features, which are in turn scored 0 (absent), 1 (improving), 2 (stable), 3 (worsening), 4 (new) according to their variation compared to the previous month. Domain scores can also be converted into quantitative scores with the following criteria: A=12; B=8; C=1; D, E=0 (Isenberg, Rahman et al., 2005, Yee, Cresswell et al., 2010). Simple disease activity scores can be combined with numerical rating scales such as the 0.0-3.0 or the 0-3 Physician Global Assessment (PGA) scale to generate composite scores.

Composite scores are increasingly employed to assess treatment response and surrogate clinical remission (van Vollenhoven, Voskuyl et al., 2017) to compensate for SLE complexity. The SLE responder index (SRI) tool incorporates variations in the BILAG scores (no new A domain and no more than one new B domain), PGA (increase in the score not exceeding 0.3 points) and SELENA-SLEDAI (decrease of at least four points) compared to a reference evaluation (Luijten, Tekstra et al., 2012). Variants of the SRI include different SELENA-SLEDAI thresholds (SRI-6, SRI-8,… as opposed to SRI-4). In a similar way, the BILAG-based composite lupus assessment endpoint (BICLA) tool defines treatment response as improved BILAG scores (all A scores turned to B, C, or D; all B scores turned to C or D) provided that no new BILAG A or no more than one BILAG B appear (see above for the SRI), no worsening in the SLEDAI score and no more than 10% worsening of the PGA score are observed (Wallace, Kalunian et al., 2014). Lupus Low Disease Activity State (LLDAS) is a third composite index surrogating disease remission (Zen, Iaccarino et al., 2018) by integrating SLEDAI-2K, PGA, treatment tolerance and occurrence of new manifestations compared to previous visits (Franklyn, Lau et al., 2016). Evidence from clinical trials and "real-life" studies suggests that LLDAS might be easily and reproducibly employed in multiple settings as a treatment endpoint (Golder, Kandane-Rathnayake et al., 2019, Ramirez, Canti et al., 2019b). Additional scores such as the Lupus Multivariable Outcome Score (LUMOS) are currently under development (Abrahamowicz, Esdaile et al., 2018).

In addition to general disease clinimetrics, other indices are used to assess activity, damage and prognosis for specific manifestations. For example, the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) is selectively used to quantitate chronic and acute mucocutaneous involvement (Klein, Morganroth et al., 2010), while the Global Antiphospholipid Syndrome Score (GAPSS) and the score developed by Petri

et al. are useful for adapting ischemic risk prediction to the setting of SLE with or without aPL (Petri, Barr et al., 2019, Sciascia, Sanna et al., 2013).

Treatments

Despite significant achievements in understanding SLE pathogenesis, only a limited set of treatments are currently available for patients with this disease. Glucocorticoids remain the mainstay of therapy to induce remission from the majority of active lupus manifestations (Fanouriakis, Kostopoulou et al., 2019, Gordon, Amissah-Arthur et al., 2018). However, a detrimental role of glucocorticoids in terms of damage accrual and risk of infectious, metabolic and cardiovascular complications even at low doses is increasingly recognised. Accordingly, a paradigm shift in the use of these drugs is currently ongoing towards the systematic use of very short-term high dose intravenous pulses followed by oral corticosteroids administered with faster tapering regimens starting from lower peak doses and ending sooner with the lowest effective amount of drug (Fanouriakis et al., 2019, Fanouriakis, Kostopoulou et al., 2020, Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021). There is, however, much more debate on the optimal timing for glucocorticoid discontinuation in patients with lower disease activity (Fanouriakis et al., 2020, Fasano, Coscia et al., 2021, Moroni, Gallelli et al., 2006, Tani, Elefante et al., 2019). Conversely, there is unanimous agreement on the use of hydroxychloroquine in all patients with SLE unless contraindicated by allergy or drug-related toxicity. This indication is supported by the excellent safety profile of this drug and the robust long-term evidence indicating its role in promoting patient survival, minimising the risk of flares after remission (Costedoat-Chalumeau, Dunogue et al., 2014, Dorner, 2010, Fasano, Pierro et al., 2017) and possibly contrast the onset of complications (Hsu, Lin et al., 2017). Similar evidence is progressively emerging for belimumab, an anti-B-cell activating factor (BAFF) monoclonal antibody with immunomodulant activity (Bruce, Urowitz et al., 2016, Furie, Petri et al., 2011, Iaccarino, Bettio et al., 2017, Manzi, Sanchez-Guerrero et al., 2012, Urowitz, Ohsfeldt et al., 2019).

A limited set of immunosuppressants are currently available to boost and maintain remission in patients with SLE. Mycophenolate mofetil (or mycophenolic acid) is largely employed for major SLE manifestations and constitutes the first-choice treatment for LN.

Cyclophosphamide is also highly potent and effective but currently less employed due to its major side effects on bone marrow, cardiac and gonadal function. Methotrexate, azathioprine, cyclosporine and, less frequently, leflunomide, tacrolimus or dapsone are also employed for the treatment of patients with SLE. Rituximab, an anti-CD20 monoclonal antibody targeting B-cells while sparing plasma-cells is also employed for remission induction in patients with severe manifestations, especially in case of refractoriness to other treatments. The use of rituximab is, however, off-label in multiple Countries, including Italy, due to controversial efficacy data. Specifically, multiple clinical trials involving the use of rituximab have failed to meet their efficacy endpoints, in contrast with evidence from "real-world" cohorts (Merrill, Neuwelt et al., 2010, Reddy, Jayne et al., 2013, Rovin, Furie et al., 2012, Terrier, Amoura et al., 2010). It is currently still unclear whether biological reasons, such as intrinsic resistance to rituximab by patients with SLE (Reddy, Cambridge et al., 2015, Reddy, Croca et al., 2013), or flaws in the design of the clinical trials could account for this incoherence.

Indeed, multiple clinical trials aiming to demonstrate the potential usefulness of novel pharmacological agents have failed over time, despite promising pre-clinical evidence. The reasons behind this disappointing series of events is, again, unclear. Failure to identify homogeneous groups of patients sharing similar pathogenic and clinical features within the broad spectrum of SLE pathophysiology might be a potential general explanation. Consistently, more recent trials aiming at addressing the efficacy of novel agents in selected disease phenotypes, as in the case of baricitinib for arthritis in SLE have shown promising results (Wallace, Furie et al., 2018). Other investigators have instead taken advantage of post-hoc analyses on previously failing studies to identify patient subpopulations more likely to respond to a given treatment and re-design treatment endpoints based on the clinical profile of these subjects. This was specifically the case of SRI (see above) in the development of the trials involving the use of belimumab. In fact, patients with serological activity (that is low complement and positive ADNA) were more likely to achieve a treatment response after belimumab when assessed through SRI. The same population is also more likely to achieve a SRI-4 endpoint due to the role of SLEDAI as the major driver of SRI-4 responses, with low complement and ADNA accounting cumulatively for four SLEDAI points (Manzi et al., 2012). More recently, two twin trials assessing the potential efficacy of anifrolumab, an interferon

inhibitor, in SLE have been completed. While the former one did not meet its SRI endpoint, the latter one was successful, being based on a BICLA endpoint, and led to the approval of the drug by the US Food and Drug Admistration (FDA) and recommended for approval by the European Medicine Agency (Furie, Morand et al., 2019, Morand, Furie et al., 2020). Additional molecules either targeting B-cell function, T cell costimulation or innate immune pathways including dendritic cell maturation and complement activation are currently under clinical and pre-clinical development.

Antigen-specific induction of immune tolerance through repetitive and dose-increasing antigen exposure is an established treatment strategy in patients with IgE-mediated allergy to inhalants or hymenopter venom (Nakagome & Nagata, 2021). Pharmacological strategies aiming to restore immune tolerance towards self antigens has less been developed in the setting of autoimmune disorders, but might be attractive, especially in terms of safety and long-term drug-related toxicities for patients with SLE, who show multiple aspects of impaired immune tolerance towards autoantigens (Robinson & Thomas, 2021). *In vitro* studies showed the potential mechanistic feasibility of histone peptides of inducing Treg cells from PBMC derived from patients with SLE (Zhang, Bertucci et al., 2013). Peptide autoepitopes bound to MHC multimers and embedded in iron oxide nanoparticles caused a selective expansion of antigen-specific regulatory T cells along with reversal of clinical manifestations in murine models of other autoimmune diseases, such as type I diabetes, multiple sclerosis and rheumatoid arthritis (either with wild-type or human transgenic HLA background) (Clemente-Casares, Blanco et al., 2016). Apparently, this approach was superior to alternative strategies based on the induction of tolerogenic antigen presenting cells through nanoparticle delivery of autoepitopes and immunomodulatory signals, which were only able to prevent, but not to treat autoimmunity in murine models (Yeste, Nadeau et al., 2012). Lupuzor, a peptide drug with potential tolerogenic properties identified in mice after screening an array of potential candidates has also been tested in a human setting. Despite promising results in phase II trials, a phase III trial with Lupuzor did not meet its endpoint (Robinson & Thomas, 2021, Wallace, 2019, Zimmer, Scherbarth et al., 2013). As with the previous cases, the reasons for this failure are unclear. Mechanistically, the drug, which derives from the U1-RNP protein, had shown potential evidence of an immunomodulatory role on autophagy and MHC class II expression by B-cells (Robinson & Thomas, 2021).

However, anti-U1-RNP immunity is not SLE-specific and studies on T cell responses against this antigens have shown disappointing results in human settings (Kattah, Newell et al., 2015) (see also below). On the other hand, failure to design appropriate trial endpoints and tools for selecting and monitoring patients for treatment responses based on their individual immune profile might also account for these results.

Aetiopathogenesis

Genetics

Nucleic acids have a central role in the pathogenesis of SLE both as target antigens and as plastic vehicles of genetic information. Extensive evidence from genome-wide association studies suggests that a multitude of polymorphisms affecting the functionality of both innate and adaptive immunity might concur to the protean clinical phenotype of SLE (**Table 1**). Notably, the majority of genetic loci associating with increased SLE susceptibility are located in non-coding regions, indicating a prominent role of dysregulated gene expression control rather than structural abnormalities in key inflammatory proteins as the pivotal mechanism accounting for inflammation in SLE (Bentham, Morris et al., 2015, Chen, Morris et al., 2017). Consistent with a significant modulating role of female sex hormones in enhancing SLE susceptibility, men with SLE show a higher number of autosomal mutations than women with SLE (Hughes, Adler et al., 2012). Patients with early-onset SLE and/or lupus nephritis also show a higher burden of risk polymorphisms for the development of SLE (Chen, Wang et al., 2020).

More recent evidence points to the existence of additional genetic factors associated with selected disease phenotypes. This specific setting of active investigation encompasses genetic loci associating not only with classical inflammatory modulators, but also with tissue/organ-specific structural proteins. Mutations affecting the functionality of these genes might, in fact, affect the ability of targets of inflammation to efficiently adapt to the inflammatory injury and/or prevent irreversible damage (Baqai, Isenberg et al., 2014, Chung, Brown et al., 2014, dos Santos, Bringhenti et al., 2015, Faria, Goncalves et al., 2017, Mohan & Putterman, 2015, Ramirez, 2018, Ramirez et al., 2015a, Ramirez, Lanzani et al., 2015b). Epigenetics also has a relevant role in SLE pathogenesis. Altered histone acetylation and methylation promotes aberrant expression of immunomodulating and enhancing genes besides affecting cell apoptosis and, ultimately,

probability of exposure of key nuclear antigens such as DNA and histones (Hu, Qiu et al., 2008). Altered angiotensin converting enzyme 2 (ACE2) epigenetics has been hypothesised as a potential risk factor for severe COVID-19 in patients with SLE (Sawalha et al., 2020).

Table 1: selected non-HLA genetic loci associated with SLE at genome-wide level of significance

Genetic locus	Functional meaning
Factors involved in innate immune responses	
IRF5	Interferon responses
IRF7	Interferon responses
IRF8	Interferon responses
TLR7	Maturation of antigen-presenting cells
Factors involved in general leukocyte biology	
ITGAM	Cell adhesion
PTPN22	B and T cell activation, interferon responses
STAT4	Interferon responses, lymphocyte activation
TNFAIP3	Reduction of lymphocyte activation through NF-kB inhibition
TYK2	Inflammatory responses downstream IL10 and interferon alpha
Factors involved in B cell and antibody responses	
BLK	B lymphocyte activation
FCGR2A	Antibody-mediated phagocytosis
FCGR2B	Antibody-mediated phagocytosis
IL10	B lymphocyte survival and maturation
Factors involved in T cell responses	
CD40	Co-stimulation
CD152 (CTLA-4)	Co-stimulation
CD80	Co-stimulation
IL12A	Th1 polarisation
TNFSF4 (OX40L)	Co-stimulation

Factors involved in the innate immune response

A first set of SLE-related genes encompasses crucial nodes in the initiation and perpetuation of type I interferon-related pathways. IFNα, in turn, constitutes the key mediator of the hallmark antiviral-like responses characterising SLE and other connective tissue diseases (see below). Interferon regulatory factors (IRF) such as IRF5 and IRF7 are involved in the control of IFNα tone and in the expression of IFNα-related genes (which, in turn, constitute the so-called *IFN-signature*). Polymorphisms in IRF genes and in molecules providing intracellular signalling downstream the IFNα receptor, such as signal transducer and activator of transcription (STAT) 1 and 4, have been linked to increased SLE susceptibility (Niewold, Kelly et al., 2008, Niewold, Kelly et al., 2012, Vaughn, Kottyan et al., 2012). Genetically determined alterations in the expression and functionality of the complement cascade and of innate pattern recognition receptors (PRR) concur with altered IFNα signalling in causing a dysfunctional processing and clearance of exogenous and endogenous antigens, including those deriving from cell death debris and/or reactivation of latently infecting viruses or endogenous retroviral elements (Lee, Lee et al., 2012, Mohan & Putterman, 2015). Among cell-bound PRR, TLRs are crucial to trigger the activation of the innate immune response. Altered TLR7 function affects nucleic acid sensing and has been linked to SLE susceptibility (Ramirez-Ortiz, Prasad et al., 2015). Interestingly, TLR7 along with other SLE-related genes is located on the X chromosome and might escape the X-inactivation programme, potentially contributing to an enhanced susceptibility to SLE among women (Sasidhar, Itoh et al., 2012). Mutations affecting the cytoskeleton have also been associated with SLE and might contribute to a defective clearance of cell death debris by phagocytes (Kim-Howard, Maiti et al., 2010, Maiti, Kim-Howard et al., 2014).

Factors involved in the adaptive immune response

Multiple genetic loci contribute to potential alterations in the adaptive immune response as observed in SLE. Class I and II human leukocyte antigens (HLAs) constitute the key mediator of antigen presentation to T lymphocytes and play an intuitively crucial role in the susceptibility to multiple immune-mediated diseases, including SLE. HLA-DRB1 gene variants have more consistently been associated with SLE susceptibility, in line with a prominent role of CD4+ T cell responses in the pathogenesis of SLE (Deng & Tsao,

2010). HLA-DRB1*03:01 and 15:01 have more robustly been associated to the development of SLE, although other variants, such as 07:01, 08:01 and 09:01 might also play a role, possibly with variable significance among distinct ancestries (Arango, Perricone et al., 2017, Bang, Choi et al., 2016, Morris, Taylor et al., 2012, Niu, Zhang et al., 2015, Shimane, Kochi et al., 2013, Teruel & Alarcon-Riquelme, 2016b). Of note, selected HLA-DRB1 variants have also been associated with specific manifestations and complications within the spectrum of SLE rather than to a general predisposing role for the development of the disease (Lundstrom, Gustafsson et al., 2013).

Besides HLA, additional genetically determined factors might contribute to dysfunctional adaptive immune responses. Protein tyrosine phosphatase N22 (PTPN22) is associated with a vast array of functions including B and T cell activation, besides regulation of myeloid-driven IFNα responses. Polymorphisms in PTPN22 have been associated with T cell mediated diseases such as anti-neutrophil cytoplasmic antibody AAV and SLE (Alberici et al., 2014, Bentham et al., 2015, Ivashkiv, 2013). Additional molecules involved in intracellular signalling downstream the B and the T cell receptor have been associated to SLE susceptibility and include the B-lymphocyte kinase (BLK), the leukocyte C-terminal/Yamaguchi sarcoma virus homologue novel tyrosine kinase (LYN) or the C-terminal Src kinase (CSK) (Mohan & Putterman, 2015). Other genetic hotspots for SLE susceptibility involved in the modulation of the adaptive response include T cell costimulatory molecules such as OX40 ligand and CD80 (Mohan & Putterman, 2015, Wang, Zhang et al., 2021). Polymorphisms in immunoglobulin Fc receptors might also prompt dysfunctional humoral adaptive responses including incomplete disposal of cell death debris (Brown, Edberg et al., 2007).

Dysfunction of the innate response

Aberrant deployment of the innate immune response is a hallmark of SLE and might play a more relevant role in the initiation of the pathogenic cascade. Defective clearance of cell death debris, either resulting from apoptosis, necroptosis, pyroptosis or, more significantly, suicidal formation of neutrophil extracellular traps (NETs) constitutes a fundamental mechanism in SLE pathophysiology (Emlen, Niebur et al., 1994, Lood, Blanco et al., 2016, Silva, Garcia et al., 2002). On the one hand, overload of cell remnants might be due to reduced cell survival. Impaired DNA damage response has been

described in SLE and might account for the pathogenic effect of environmental stimuli such as ultraviolet light or endogenous factors such as latent virus or retroviral elements in triggering disease flares (Khadjinova et al., 2021, Sander, Szabo et al., 2005, Souliotis & Sfikakis, 2015, Trela, Nelson et al., 2016).

NET formation is also a characteristic feature of SLE (Manfredi, Covino et al., 2015, Manfredi, Rovere-Querini et al., 2010). NETs are decondensed chromatin threads of nuclear or mitochondrial origin endowed with a vast array of microbicidal moieties and evolutionarily linked to host defence. Components of NETs include digestive enzymes such as myeloperoxidase, PRR enhancing pathogen opsonisation and pro-coagulant mediators such as tissue factor. Accordingly, exposure of nuclear antigens and pro-thrombotic factors in a pro-inflammatory environment not only promotes the generation of autoimmunity but also may trigger the coagulation cascade (a process termed immunothrombosis in this context) (Engelmann & Massberg, 2013, Mantovani, Cassatella et al., 2011, Villanueva, Yalavarthi et al., 2011).

Upstream factors involved in inducing neutrophils to form NETs are incompletely understood (Manfredi, Ramirez et al., 2018). Platelets constitute key functional partners of neutrophils, besides their haemostatic role. Aberrant interactions between platelets and neutrophils, therefore, constitute a major trigger of neutrophil activation and a promising inciting factor for the development of NETosis (Joseph, Harrison et al., 2001, Maugeri, Cattaneo et al., 2014). Enhanced neutrophil survival possibly in combination with overactive autophagy cell programmes might constitute an additional favouring factor for NETosis and account for the frequent detection of large dense granulocytes in patients with SLE (Maugeri, Campana et al., 2014, Tang, Zhang et al., 2015, Villanueva et al., 2011). Quantitative or qualitative impairment of scavenger mechanisms constitutes a potential additional factor involved in defective elimination of nuclear components and pro-inflammatory mediators resulting from cell death in the setting of SLE. Innate PRR such as pentraxins have a role in opsonising pathogens and self components promoting their clearance and are defectively deployed in SLE. This mechanism might possibly also account for the typical low C-reactive protein levels observed clinically in patients with SLE, despite active inflammation. Low complement levels either due to genetic causes or to extensive antibody responses are also a clinical hallmark of active SLE and might contribute to inefficient humoral innate responses (Chen, Daha et al., 2010, Fischetti &

Tedesco, 2006, Leffler, Martin et al., 2012, Liszewski & Atkinson, 2011, Tsang, Bultink et al., 2017). In addition, inefficient DNA degradation due to genetically determined or acquired DNAse deficiency has also been described in SLE and may contribute to facilitated sensitisation to nuclear components (Radic, Herrmann et al., 2011, Shin, Park et al., 2004).

Enhanced antiviral-like IFNα-driven responses constitute the second pillar of innate immune dysfunction in SLE. Strikingly, neutrophil undergoing NETosis are interferogenic as they exert an activating effect on plasmacytoid dendritic cells (Garcia-Romo et al., 2011, Swiecki & Colonna, 2015). IFNα, in turn, promotes myeloid dendritic cell activation facilitating (auto)antigen presentation and downstream adaptive responses. Specifically, IFNα promotes plasmablast expansion and enhances antibody responses, promoting persistence of inflammation (Jego, Palucka et al., 2003, McKenna, Beignon et al., 2005, Swiecki & Colonna, 2015). Interestingly, IFNα-driven responses also contribute to endothelial dysfunction and stimulate endothelial-derived microparticle release, thus contributing to systemic spread of pro-inflammatory signals and promoting tissue infiltration by circulating leukocytes (Dieker, Tel et al., 2016, McCarthy, Moreno-Martinez et al., 2017, Ostergaard, Nielsen et al., 2017, Tyden, Lood et al., 2017). As anticipated, IFNα-targeted diagnostic and therapeutic strategies have been tested in lupus cohorts. Disappointingly, IFNα level tracking demonstrated limited practical value in patient monitoring (Banchereau et al., 2016) and clinical trials with IFNα inhibitors had controversial results (Furie, Khamashta et al., 2017, Khamashta et al., 2016, Morand et al., 2020). Recent research in the setting of anti-SARS-CoV-2 responses provides reconciling evidence: anti-IFNα antibodies might be part of the serological spectrum of SLE and cause improper, rather than simply enhanced, antiviral responses during sterile and non-sterile inflammation (Banchereau et al., 2016, Gupta et al., 2021a, Gupta et al., 2021b, Sawalha et al., 2020). Impaired B regulatory cell differentiation in the absence of low-grade tonic IFNα release constitutes a potential mechanism linking dysregulated IFNα-responses to humoral autoimmunity in SLE (Menon, Blair et al., 2016).

While alterations in platelet-neutrophil, neutrophil-dendritic cell and neutrophil-endothelium axes have an established role in the pathogenesis of SLE, emerging evidence from animal models and human studies suggests the potential contribution of additional cellular innate immune players to the pathogenesis of SLE. In particular, mast cells have

been shown to participate in lupus skin inflammation and might have a role in modulating T cell function through inhibition of T regulatory responses and promotion of antigen presentation by dendritic cells (Kaczmarczyk-Sekula, Dyduch et al., 2015) (Walker, Hatfield et al., 2012). More intriguingly, enhanced activation of the basophil-IgE axis in the setting of Th2-driven responses has recently been claimed as a distinct but significant pathway in the pathogenic network of SLE, besides its conventional role in allergic inflammation (Charles, Hardwick et al., 2010). Data from lupus-prone mice suggest that basophils contribute to B cell responses by releasing pro-B cytokines such as IL4, IL6 and BAFF in the context of secondary lymphoid organs. Evidence of basophil activation in human SLE has also been reported (Dema & Charles, 2014, Dema, Lamri et al., 2017, Henault, Riggs et al., 2016, Pan, Feng et al., 2017, Pellefigues & Charles, 2013).

Dysfunction of the adaptive response

Humoral responses

Autoantibodies play a central role in the pathogenesis of SLE and constitute the mainstay of lupus diagnostic tools besides complement factor measurement. Consistently, transcriptome analyses have shown a robust correlation between plasmablast signature and disease activity (Banchereau et al., 2016). Deposition of antibodies at sites of inflammation with eventual complement activation, leukocyte recruitment and non-resolving inflammation is regarded as the principal cause of direct tissue and organ damage in patients with SLE (Tsokos, 2011). Limited evidence also suggests the potential existence of cell-penetrating antibodies causing non-conventional cell cytotoxicity events and possibly contributing to exacerbated DNA damage responses in SLE (Portales-Perez, Alarcon-Segovia et al., 1998, Zannikou, Bellou et al., 2016). To this regard, some authors also hypothesised a role for these antibodies in shaping the unique susceptibility profile of patients with SLE towards cancer, with frequent solid tumours being relatively less frequent and haematological malignancies more frequent in SLE compared to the general population (Portales-Perez et al., 1998, Zannikou et al., 2016). In addition, autoantibodies may facilitate DNA internalisation by dendritic cells through FCγIIra, favouring TLR activation and IFNα responses (Bave, Magnusson et al., 2003). Evolutionarily, this mechanism has possibly developed to enhance host response to viruses and might therefore be implicated in the link between infections and lupus flares (Henault et al.,

2016, Joo et al., 2021). Interestingly, anti-DNA class E immunoglobulins are potent inducers of IFNα release by plasmacytoid dendritic cells and might act in synergy with canonical anti-DNA IgG in promoting inflammation. Auto-IgE also correlate with SLE activity possibly supporting a pathophysiological view of SLE as a multilevel dysfunction of the immune system, centred on aberrant nuclear antigen processing but extending to a multitude of inflammatory pathways, including those conventionally associated with anti-parasite or allergic responses (Atta, Santiago et al., 2010, Dema, Pellefigues et al., 2014, Henault et al., 2016). Therapeutic depletion of total IgE showed evidence of possible clinical benefit in patients with SLE (Hasni, Gupta et al., 2019). Besides autoantibody production, B cells can contribute to enhanced autoimmune responses as antigen presenting cells.

Multiple factors might account for dysregulated B cell function in SLE. Female sex hormones enhance B cell responses and increase B cell selection permissibility to autoantigens, possibly accounting for the characteristic demographic profile of SLE (Crispin & Tsokos, 2015). Myeloid (Dema et al., 2017, Scapini, Hu et al., 2010) or T cell-derived B-stimulating soluble factors such as BAFF or a proliferation inducing ligand (APRIL) contribute to B cell activation and rise in correlation with active disease and B-cell depletion failure in patients with SLE (Carter, Isenberg et al., 2013, Salazar-Camarena, Ortiz-Lazareno et al., 2016, Zhao, Li et al., 2010), thus constituting a successful therapeutic target (Stohl, 2014).

T-cell-mediated responses

TCR signalling

T cells are endowed with antigen recognition selectivity at a molecular level, fine-tune the inflammatory response with extreme inter- and intra-individual variability and likely have a non-redundant role in the protean clinical/pathophysiological complexity of SLE. Nonetheless, T cell-mediated responses have less been investigated compared to B cell and antibody-related mechanisms (Rother & van der Vlag, 2015). As anticipated, TCR-MHC engagement constitutes the molecular correlate of specific antigen recognition by T cells and occurs thanks to the contribution of a vast array of ancillary mediators both at the level of the cell surface and intracellular space. In particular, functional TCR signalling requires interactions with the lineage-specific CD3 complex, which, in turn, is

physiologically composed of four protein subunits (gamma, delta, epsilon and zeta). Secondary to genetic or epigenetic factors (Chowdhury, Tsokos et al., 2005), patients with SLE are characterised by low levels of CD3ζ (Pang, Setoyama et al., 2002), which is structurally and functionally substituted by the common gamma subunit of the immunoglobulin Fc receptor (FcRγ). This latter cell surface protein, in turn, promotes stronger downstream inflammatory signals, consistent with its physiological selective expression on activated effector T cells, besides taking part in the formation of the high-affinity IgE receptor (Katsuyama, Tsokos et al., 2018).

T cell subpopulations

Although CD8+ T cell counts correlate with disease activity scores (Kubo, Nakayamada et al., 2017) and might play a role in events such as neuronal damage (Contin-Bordes, Lazaro et al., 2011, Liblau, Gonzalez-Dunia et al., 2013) or nephritis (Zabinska, Krajewska et al., 2016), alterations in CD4+ subpopulations have been more robustly detected in patients with SLE. In line with the existence of abnormalities in multiple aspects of the immune and adaptive response in SLE, all CD4+ subpopulations likely contribute to the initiation and persistence of chronic inflammation and expand or contract dynamically according to the relapsing/remitting nature of the disease. In clinical practice, low lymphocyte and, more specifically, low CD4+ counts constitute routine findings in patients with SLE, especially during active disease (Carli et al., 2015). Pathophysiologically, decreased lymphocyte count might represent enhanced migration into target tissues, exaggerated apoptosis rates or the effect of autoimmune phenomena targeting lymphocytes (Abdirama, Tesch et al., 2021, Dolff, Abdulahad et al., 2010, Fayyaz, Igoe et al., 2015, Li, Harada et al., 2007).

Patients with SLE show abnormal patterns of T cell differentiation into memory cells (Fritsch, Shen et al., 2006, Sen, Chunsong et al., 2004), although SLE heterogeneity may prevent appreciation of numerically significant differences with the reference general population when small sample sizes are considered (Maldonado, Mueller et al., 2003). According to some authors, expansion of CD4+ effector memory cells is a characteristic feature of patients with active disease (Piantoni, Regola et al., 2018). Other authors reported lower to normal T_{EM} and T_{CM} levels in patients with SLE, suggesting that only the fraction of these T cell subset expressing selected costimulatory molecules might

identify patients with active disease (Zhou, Hu et al., 2018). Expansion of other T cell subpopulations identifying patients subset at increased risk for cardiovascular complications has also been reported (Baragetti, Ramirez et al., 2017). More recently, evidence has been provided suggesting that total CD4+ and CD8+ T_{SCM} cells are expanded in patients with SLE and able to efficiently repopulate the host Tfh repertoire (Lee, Park et al., 2018). These data corroborate data from other rheumatologic settings (Cianciotti, Ruggiero et al., 2020) and provide potential clues on the mechanisms underlying a) persistence of inflammation and immunological memory towards target autoantigens despite treatments including B cell depletion; b) maintenance of plasticity in terms of antigen recognition and downstream functional differentiation leading to 1) facilitated sensitisation to antigen showing molecular mimicry with the original stimulus; 2) intraindividual variability in terms of clinical phenotypes induced by the same autoreactive pathway (Salem, Subang et al., 2015).

A numerical deficit in Treg cells has been observed by multiple authors in patients with active disease (Lee, Hong et al., 2008, Piantoni et al., 2018, Yang, Chu et al., 2009). In apparent contrast, other authors found higher Treg counts in patients with SLE, with enrichment of Treg-skewed responses in selected patient clusters, independent on disease activity (Kubo et al., 2017). At the opposite extreme of the spectrum, some authors found evidence of increased Treg counts in patients with active disease (Bonelli, Goschl et al., 2014). Methodological discrepancies in gating strategies for T cell classification, disease activity measurement and lack of repeat assays to control for interindividual variability might account for these diverging results (Zhang, Ma et al., 2018). In addition, numerical variations in the proportion of Treg might be uncoupled from their biological functionality (Bonelli, von Dalwigk et al., 2008). Activity-associated B regulatory cell expansion has also been reported (Yang, Yang et al., 2014). Enhanced Th2 and Tfh(2) responses are intuitively linked to dysregulated antibody production as observed in SLE. Higher levels of Tfh have consistently been detected in patients with SLE both in blood and affected tissues (Kubo et al., 2017, Yang et al., 2014). Th2-inbalanced responses have also been described (Funauchi, Ikoma et al., 1998). Furthermore, variations in the Th1/Th2 have been suggested to correlate with specific clinical phenotypes, especially in the setting of lupus nephritis (Miyake, Akahoshi et al., 2011). Th17 role in SLE is growingly appreciated. Numerical expansion of Th17 cells has been reported by multiple

authors (Shah, Lee et al., 2010, Yang et al., 2009, Zhong, Jiang et al., 2018) consistent with increased circulating levels of IL17 (Vincent, Northcott et al., 2013, Zhou et al., 2018). Nonetheless, other studies did not replicate these results (Kubo et al., 2017). Animal models of SLE suggest that IL17 dominates the inflammatory landscape in response to critical SLE target antigens (Kattah et al., 2015, Summers, Odobasic et al., 2014). Pharmacological blockade of IL17 responses has shown promising results in recent trials (van Vollenhoven, Hahn et al., 2018).

Antigen-specific T cells

Very limited evidence exists regarding the nature and behaviour of antigen-specific T cells in SLE. The majority of studies employed peptide libraries from known SLE protein target antigens to stimulate patient or mouse lymphocytes and observe T cell proliferation, expression of cell surface markers of activation and/or release of IFNγ and other cytokines. Chromatin-specific responses have more consistently been reported in the literature in human settings (Bruns, Blass et al., 2000, Lu, Kaliyaperumal et al., 1999), followed by indirect evidence of Sm T cell autoreactivity (Talken, Schafermeyer et al., 2001, Zhao, Ren et al., 2019b). More recent studies found that nuclear antigen-reactive T cells can be detected in blood and urine of patients with SLE, correlate with disease activity and associate with renal involvement (Abdirama et al., 2021, Tesch, Abdirama et al., 2020). Interestingly, similar to data from penicilloylated peptide-stimulated cells (Azoury et al., 2018, Nhim et al., 2013), non-negligible T cell responses were found also in healthy individuals, suggesting the existence of qualitative differences in the behaviour of autoreactive or allergen-reactive T cells between subjects with vs without immune-mediated manifestations rather than simple central tolerance factors (Abdirama et al., 2021). Potential associations among anti-Ro, anti-La responses and disease activity have also been suggested, while more controversial results exist for RNP reactivity (Abdirama et al., 2021, Kattah et al., 2015, Monneaux, Hoebeke et al., 2005), also taking into consideration that RNP is not a SLE-specific antigen from a clinical perspective. Contin-Bordes et al. employed direct *ex vivo* T cell visualisation techniques with class I MHC tetramers to identify myelin autoreactive CD8+ cells in patient with NPSLE (Contin-Bordes et al., 2011). A pilot study on patients with APS also identified β2GPI reacting CD4+ T cells adding evidence to a model where sensitisation towards this protein

promotes antigen spreading and progressive accrual of additional autoantibodies and enhancement of local inflammatory responses (Benagiano, Borghi et al., 2019, de Moerloose, Fickentscher et al., 2017, Leu, Lee et al., 2019, Salem, Subang et al., 2018, Salem et al., 2015).

AIM OF THE WORK

SLE is a complex autoimmune disease characterised by the combination of multiple defects in the physiological deployment of the immune response. Pathophysiological complexity is mirrored by extreme clinical intra- and inter-individual variability and scarcity of accurate and flexible tools for patient stratification and individualised treatments. Current strategies for monitoring and classifying patients are currently based on a limited number of serological markers which might not entirely reflect SLE clinical manifestations. To this regard, the occurrence of infectious events as disease triggers and of hypersensitivity reactions as complications of SLE has been suggested in the literature but no evidence exists linking the three aspects of immune dysfunction (that is autoimmunity, allergic diathesis and immunodeficiency) in SLE nor providing clues on the potential underlying mechanisms. Multiple key factors in the pathophysiology of SLE, including IFNα and B cell-driven responses, have been tested for their performance as markers of disease activity and therapeutic targets, leading to controversial results. T lymphocyte dynamics have less been studied in SLE, despite their putative role in promoting and maintaining autoimmunity, besides possibly promoting aberrant responses towards innocent environmental stimuli and pathogens. In particular, little is known about the existence and potential clinical and pathophysiological significance of antigen-specific CD4+ T cells in SLE. In an effort to identify novel tools for disease monitoring and potential future mechanisms for molecularly targeted treatments, this study aims to a) seek evidence of clinical correlations among autoimmune, allergic and infectious manifestations b) test if antigen-specific T cells recognising clinically relevant autoantigens, allergens and/or microbial antigens are specifically detected in the blood of patients with SLE and functionally able to respond when challenged with these antigens; c) assess whether differentiation and functional characteristics of autoantigen-specific T cells correlate with SLE clinical phenotype in terms of disease activity.

RESULTS

Clinical evidence

General features

Two-hundred-twenty-two patients with SLE, including 191 women (86%) and 31 men (14%) were cross-sectionally enrolled and prospectively followed up (see also Methods). Their general disease features are reported in **Table 2**. Most patients (135/222, 61%) had been diagnosed with SLE more than 10 years before, while 16 (7%) had less than two years of disease duration. Joint involvement (arthralgia, non-erosive or erosive arthritis), haematological and mucocutaneous manifestations were the most frequent clinical features in patients' history. Anti-double-stranded DNA antibodies were the most frequent serological feature, having been found in 170 (77%) patients. Antiphospholipid antibodies (aPL) were part of the serological profile of 45% of patients. Anti-Smith antibodies (aSm) had been consistently detected in 21% of the patients. Anti-ribonucleoprotein (aRNP), anti-Ro (aSSA) and anti-La (aSSB) antibodies were less prevalent.

At time of enrolment, most patients (135/222, 61%) were in remission according to the lupus low disease activity state (LLDAS) criteria. Accordingly, the median (interquartile range, IQR) SLEDAI-2K score was 4 (2-5). 43% of patients had irreversible dysfunction in one or more tissues or organs as estimated by SDI. One-hundred-twenty-two patients (55%) were taking prednisone at time of enrolment, with a median dose of 5 (4-7) mg/day. Ninety-three patients (42%) were taking one or more immunosuppressants (including cyclophosphamide, mycophenolate mofetil, azathioprine, cyclosporine A, tacrolimus, methotrexate, or leflunomide). One-hundred-eighty-seven patients (85%) were taking hydroxychloroquine. Regarding treatment history, 57 patients (26%) had ever been treated with methotrexate, 97 (44%) with mycophenolate mofetil, 125 (56%) with azathioprine, 39 (18%) with cyclosporine A, 57 (26%) with cyclophosphamide, 15% (7%) with rituximab and 18 (8%) with belimumab.

Table 2: clinical features of patients with SLE

Clinical features	Measurement
Females: n (%)	191 (86)
Age at onset (years): median (IQR)	28 (20-36)
Age at enrolment (years): median (IQR)	44 (34-53)
Disease duration (years): median (IQR)	13 (6-23)
Main clinical manifestations during the disease course: n (%)	
Mucocutaneous manifestations	145 (65)
Malar rash	42 (19)
Photosensitivity	40 (18)
Oral ulcers	28 (13)
Urticaria	11 (5)
Joint disease	168 (76)
Neuropsychiatric involvement	
ACR criteria	42 (19)
SIR criteria	30 (14)
Haematological manifestations	166 (75)
Haemolytic anaemia	30 (14)
Thrombocytopenia	44 (20)
Leukopenia	117 (53)
Lupus nephritis	79 (36)
Serositis	70 (32)
Pleuritis	37 (17)
Pericarditis	37 (17)
Peritonitis	2 (1)
Vasculitis	20 (9)
Constitutional symptoms	
Fever	31 (14)
Lymphadenopathy	28 (13)
Miscellanea	
Eye disease	7 (3)
Myopathy	5 (2)
Autoimmune hepatitis	12 (5)
Lung involvement	5 (2)
Serology	
ADNA	170 (77)
aSm	46 (21)
aSSA	15 (7)
aSSB	13 (6)
aRNP	39 (18)
aPL	99 (45)

Tripartite associations among disease activity, allergy and infections

Retrospective analysis

Epidemiology of allergy

This first part of the study was mainly focused on allergic events. At enrolment, 107 patients (48%) reported a history of allergy (**Table 3**). This prevalence was higher than expected from data obtained in the Italian general population according to the Italian National Institute for Statistics (ISTAT; reported prevalence of allergy = 10.7%; $p<0.001$ by binomial test) and the European Institute for Statistics (Eurostat; reported prevalence of allergy excluding asthma for Italy = 15.2; $p<0.001$ by binomial test). Immediate-type hypersensitivity reactions were most frequent (87%) among patients with allergy history. Nine patients had anaphylaxis, yielding an incidence rate of anaphylaxis of $9.1/10^4$ person-years (95% CI = 4.4-16.7), which is in line with the $10.3/10^4$ person-years incidence rate reported by Tejedor-Alonso et al. (Tejedor Alonso, Moro Moro et al., 2012) in the Spanish general population (p=NS by binomial test) and lower than the $36/10^4$ person-years anaphylaxis incidence rate reported in the Emergency Department setting for the city of Milan, Italy (Pastorello, Rivolta et al., 2001)($p<0.001$ by binomial test).

The point prevalence of anaphylaxis at time of enrolment was 4%, which is higher than expected in the Italian general population (Quercia and Incorvaia et al., 2012: 1.6%; $p=0.010$ by binomial test). Eighty-one patients with SLE reported a history of drug allergy, which accounted for more than 76% of the total allergy spectrum. The prevalence of drug allergy was higher than expected in the general population as per Wong et al. (Wong, Seger et al., 2019) who reported a 13.8% drug-allergy prevalence in the USA population compared to 36% drug allergy among patients with SLE in this study ($p<0.001$ by binomial test). 56 patients (25%) had allergy history when drug-related hypersensitivity events were excluded. This figure was higher than expected in the Italian general population according to Quercia and Incorvaia (Quercia, Incorvaia et al., 2012), who reported a prevalence of allergy of 16.5% excluding drug-allergy ($p=0.001$ by binomial test). There was no significant difference in comparison with other European populations (Langen, Schmitz et al., 2013) (**Appendix 1**).

Among drugs, antibiotics (42%) and specifically beta-lactams (32%) were the most frequent culprit agents. Thirty-three patients had respiratory allergy (prevalence 15%) and nine had allergic asthma (prevalence 4%), although none had allergic asthma as the only

allergic manifestation. The prevalence of respiratory allergy but not of allergic asthma was higher than expected in the Italian general population (Quercia et al.: 6%; p<0.001). Hymenopter venom allergy had a similar prevalence in our SLE cohort than in the Italian general population.

Table 3: allergy features in patients with SLE

	N	% of SLE	% of allergic SLE
Any history of allergy	107	48%	100%
Timing			
Immediate-type hypersensitivity	93	42%	87%
Delayed-type hypersensitivity	18	8%	17%
Both manifestations	13	6%	12%
Clinical features			
Skin manifestations	71	32%	66%
Urticaria/angioedema	69	31%	64%
Atopic dermatitis	2	1%	2%
Contact allergic dermatitis	11	5%	10%
Respiratory allergy	33	15%	31%
Allergic rhinitis	27	12%	25%
Allergic asthma	9	4%	8%
Anaphylaxis	9	4%	8%
Culprit agents			
Drugs	81	36%	76%
Antibiotics	45	20%	42%
Beta-lactams	34	15%	32%
NSAIDs	18	8%	17%
Immunosuppressants	6	3%	6%
Hydroxychloroquine	12	5%	11%
Other	50	23%	47%
Inhalants (grass, dustmites, pollens…)	32	14%	30%
Food	10	5%	9%
Hymenopter venom	2	1%	2%

Timing and clinical features of allergic events

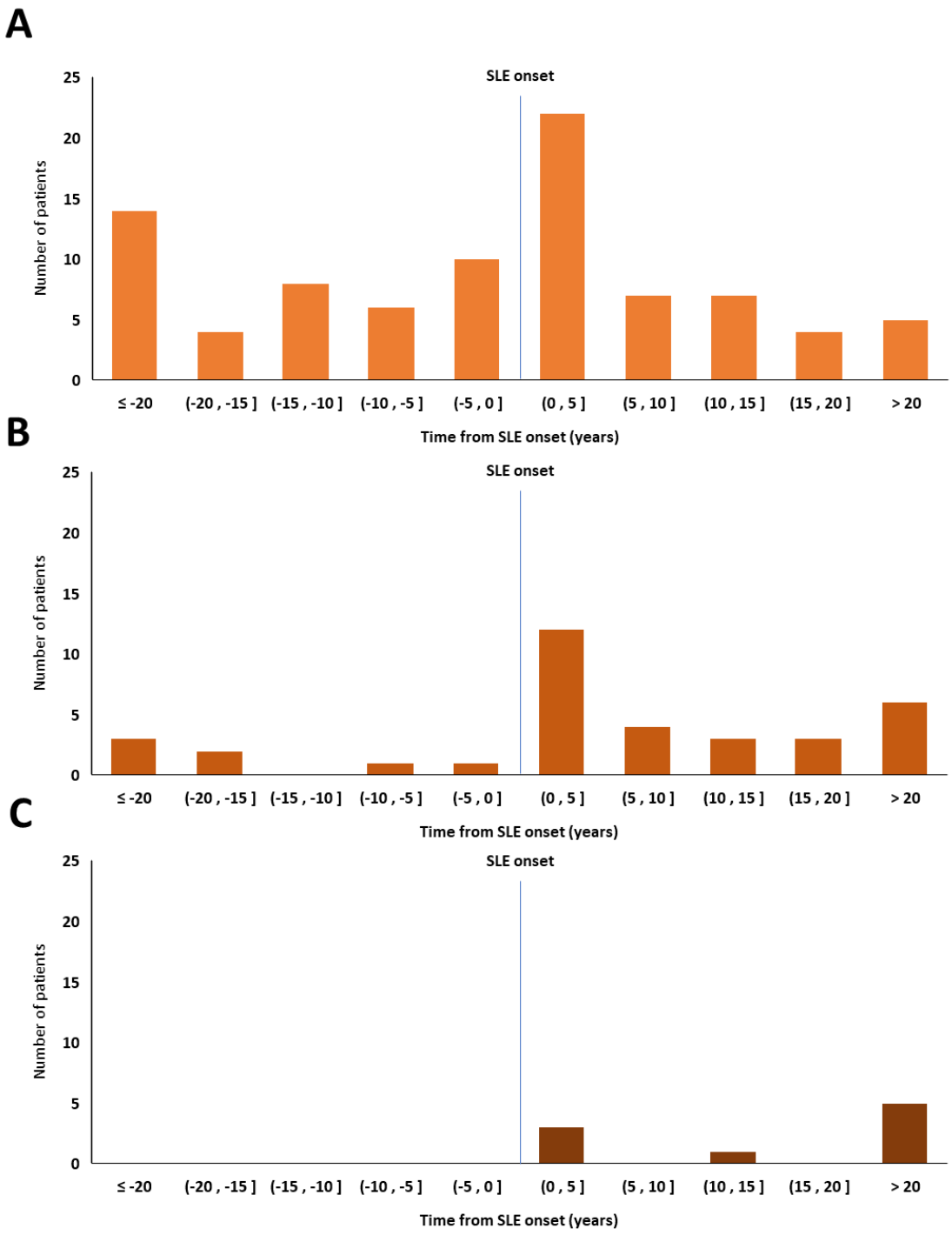
In addition to the 107 patients reporting at least one allergic event, 35 patients reported a second allergic reaction and nine a third allergic event. The median (IQR) time from SLE onset to the onset of the first allergic event was 7.55 (0.91-15.75) years (N=202). Forty-four patients (41% of patients with allergy) had their first allergic manifestation occurring after the onset of SLE. The timing of onset of the first allergic manifestation

tended to cluster around SLE onset, while the second and third manifestations occurred more frequently after disease onset (**Figure 2**). In fact, while the onset of the first allergic manifestation had an incidence rate of 0.17 (95% CI 0.12-0.24) cases per 10 person-years after more than 10 years of disease course or more than 10 years before its onset, the incidence rate of the same event within the 10 years preceding or following SLE onset was 1.52 (1.17-1.93) cases per 10 person-years ($p<0.001$). Drug-related hypersensitivity events were more frequent in patients with allergy onset after SLE (93%) than in patients with allergy onset before SLE (31%; $\chi^2=45.210$; $p<0.001$). In patients with allergy onset after SLE, a history of allergy was more frequent in patients having ever been treated with cyclosporine A (58% vs 32% in the remainder patients; $\chi^2=7.823$; $p=0.007$) or rituximab (75% vs 33% in the remainder patients; $\chi^2=8.428$; $p=0.009$).

Co-occurrence of infections with allergy was reported for 20/82 (24%) first, 11/33 (33%) second and 3/9 (33%) third allergic events with available data. SLEDAI-2K exceeding four points at time of allergy reaction increased over time as it was reported in 23/82 (28%), 10/33 (30%) and 5/9 (56%) first, second and third episodes, respectively (**Table 4**). Allergy and active SLE manifestations occurred at these time-points despite the fact that 12/107 (11%), 9/35 (26%) and 4/9 (44%) patients with a first, second and third episode respectively were taking a prednisone dose above 5 mg/day.

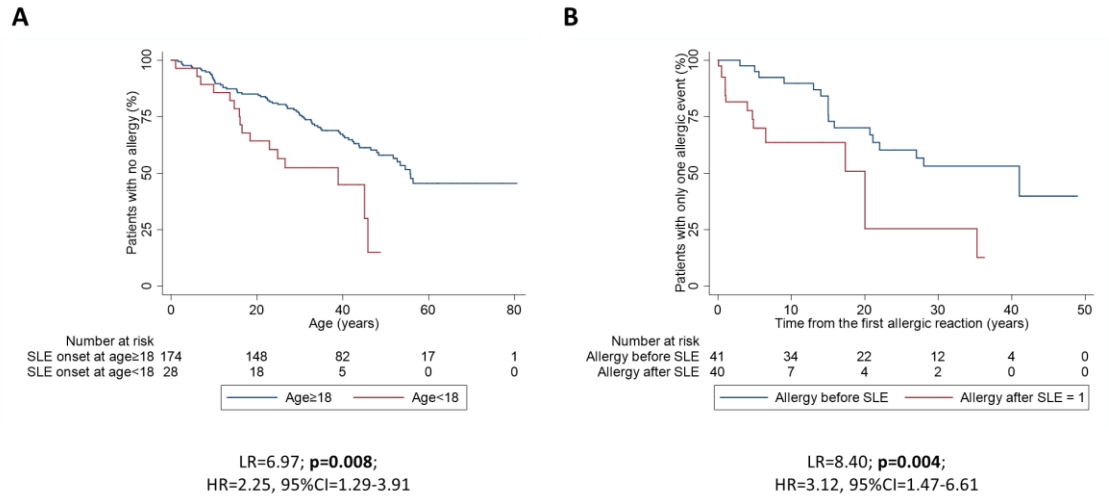
Patients with juvenile SLE (defined as SLE with onset before the age of 18) became allergic more frequently and earlier than patients with SLE onset after 18 years of age (Log rank=6.97; p=0.008; Hazard ratio, HR, =2.25, 95% CI=1.29-3.91). Patients with a first allergic event after the onset of SLE were in turn more likely to become sensitised to a second allergen and to experience a second allergic reaction earlier than patients with allergy onset before SLE (Log rank=8.40; p=0.004; HR=3.12, 95% CI=1.47-6.61; **Figure 3**). There was a statistically non-significant trend towards a higher risk of allergy in women (p=0.057 by Cox's regression analysis) and in patients with vasculitic manifestations (p=0.067 by Cox's regression analysis). Patients with a history of serositis (either pleuritis, pericarditis and/or sterile peritonitis) were slightly less likely to have a history of allergy (Log rank=4.21; p=0.040; HR=0.61, 95% CI=0.38-0.99). There were no other clinical features of SLE showing a differential distribution among patients with or without concomitant allergy. Anti-Smith antibodies showed a possible weak association with allergy (Log rank=4.01; p=0.045; HR=1.64; 95% CI=1.03-2.62).

Figure 2: timing of onset of allergy in relation to SLE onset



Histograms showing the timing of onset of the first (A), second (B) and third (C) allergic event in patient history in relation with SLE onset (blue central line). Each bar represents the number of patients with allergy occurring in a given timeframe before (left of the blue line) or after (right of the blue line) the onset of SLE. The onset of allergy (panel A) was more frequent around or after the onset of SLE. Subsequent allergic events tended to be more frequent after the onset of SLE (panel B and C).

Figure 3: onset of the first and second allergic reactions in patients with SLE



Kaplan-Meier survival curves showing the kinetics of onset of the first (A) and second (B) allergic events in patients with SLE. Patients with early-onset SLE (aged <18 years at disease onset, red line, panel A) had increased chances of becoming allergic compared to patients with later onset. Patients who were diagnosed with allergy after the onset of SLE (red line, panel B) were more likely to experience an additional allergic event during their history.

Damage accrual at time of enrolment did not differ between patients with or without allergy history. There was also no difference in SDI score accrual rate over time between patients with or without at least one allergic reaction. However, patients with a history of two or more allergic events had a higher annual rate of damage accrual (median=0.043 SDI points per year, IQR=0-0.112) compared to patients with only one allergic event (median<0.001 SDI points per year, IQR 0-0.072; p=0.046). There were no significant differences between patients with less vs at least three allergic events.

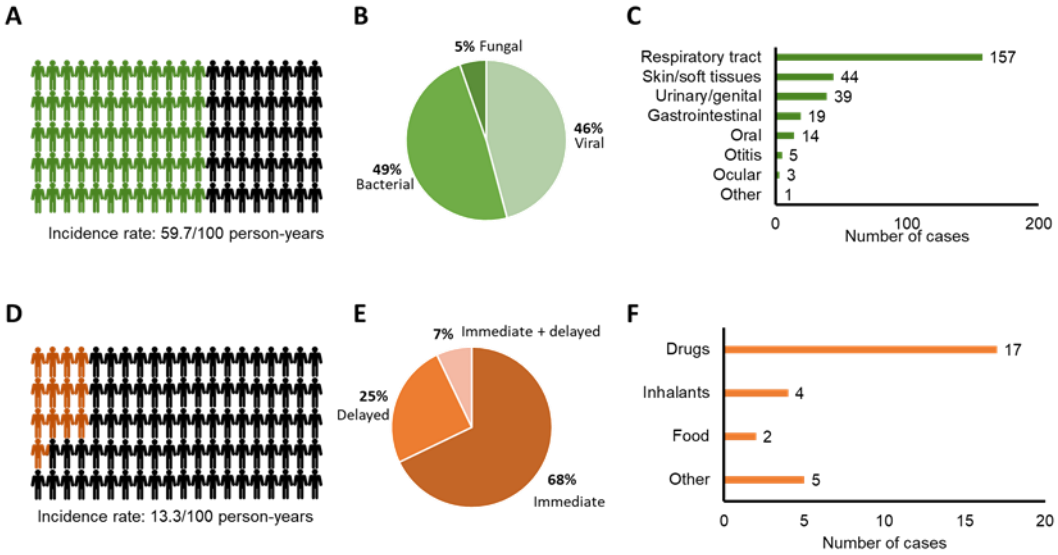
Prospective analysis

One-thousand two-hundred sixty-seven visits (459 cross-sectional visits, 808 follow up visits) were analysed. The median (IQR) interval between each visit was 5.29 (3.45-5.98) months. There were 282 infectious events requiring systemic antimicrobial therapy or absence from work over 472.40 person-years of observation, yielding an incidence rate of 59.7 (95% CI = 53.0-67.0) cases per 100 person-years. The average frequency of recent infection reporting was 18% per patient per visit. The most frequent site of infection was the respiratory tract (55%), followed by skin and mucosal infections (16%) and urinary tract/genital infections (14%). Of 168 infections with definite aetiology, 82 were bacterial

(49%) and nine fungal (5%). There were 77/168 (46%) viral infections including 23 herpesvirus infections (30% of the viral infections, 14% of all infections with definite aetiology (**Figure 4 A-C**).

Twenty-eight allergic reactions (17/28 drug-related events, 3/28 due to beta-lactam drug sensitisation) were recorded over 211.17 person-years, yielding an incidence rate of 13.26 (95% CI = 8.98-18.91) total cases and 8.05 (95% CI = 4.85-12.63) drug-related events per 100 person-years. The average frequency of recent allergy reporting was 3% per patient per visit. Nineteen reactions were of immediate-type only, seven were of delayed-type only and two showed clinical features of both immediate- and delayed-type hypersensitivity (**Figure 4 D-F**). Antibiotics and immunosuppressants (each accounting for 29% of drug-related allergic reactions) along with NSAIDs (12% of drug allergy events) constituted the most frequent culprit drugs.

Figure 4: infections and allergic reactions



Descriptive graphs showing the epidemiological features of infectious (green, panel A-C) and allergic (orange, panel D-F) events in a prospective study involving 222 patients over 1,267 consecutive visits. Panel A and D show the estimate number of infections (A) and allergic reactions (D) occurring in a year for every 100 persons considered. Panel B depicts the distribution of infectious events by aetiology. Panel C reports the site distribution of the recorded infectious events. Panel E shows the proportion of immediate-, delayed- and combined type of allergic reactions over the total of allergic reactions. Panel F depicts the number of allergic events by culprit agent.

In 795/1,267 visits (63%), patients fitted the criteria for LLDAS. The average frequency of SLEDAI-2K exceeding four points was 21% per patient per visit. The

calculated combined probabilities of observing active disease, recent infections and/or recent allergic reactions retrospectively and prospectively are reported in **Table 4**. In order to test the negative hypothesis that hypersensitivity reactions, infections and disease activity occurred as independent events, the expected probability of observing a combination of two or three events together was calculated as the product of the observed frequencies of each event at prospective analysis. These figures were then compared to the actual frequencies of occurrence of two or three combined events with the binomial test of hypotheses. The observed frequencies of combined autoimmune, allergic and/or infectious manifestations were higher than expected by considering each item independently, both at prospective and retrospective observation.

Table 4: observed and expected frequencies of allergic, infectious and autoimmune manifestations in patients with SLE

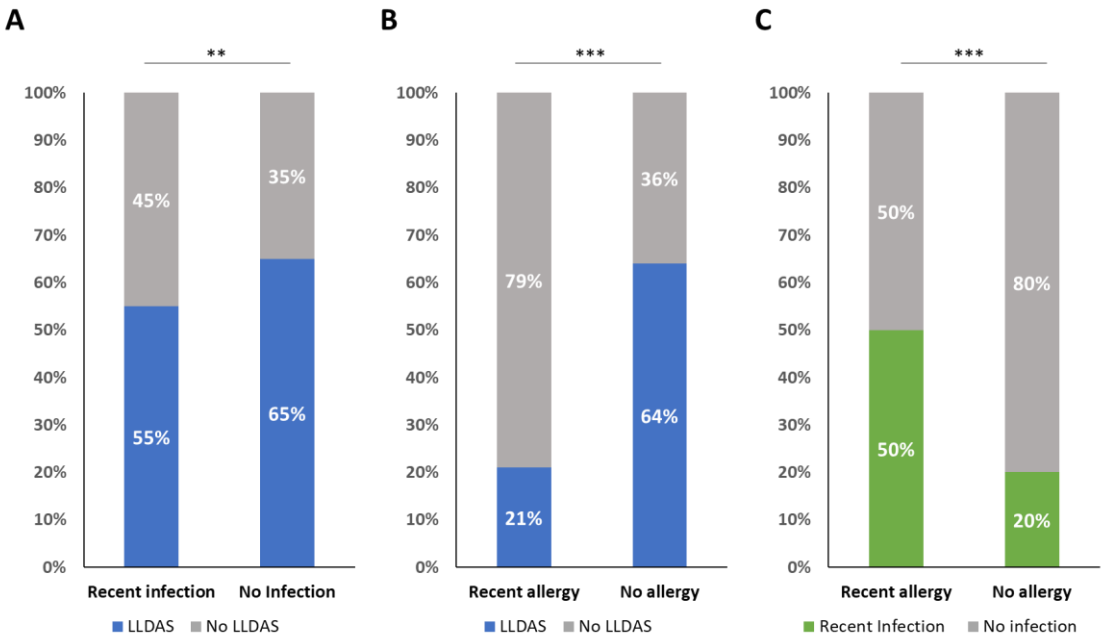
	Observed		**Expected**
	Retrospective observation	**Prospective observation**	
SLEDAI-2K>4	ND	21%^	
Recent infection	ND	18%^	NA
Recent allergy	ND	3%^	
SLEDAI-2K>4 + Recent infection	ND	4.12%	3.78%§
SLEDAI-2K>4 +Recent allergy	1st event: 23/202 (11%)*** 2nd event: 10/67 (15%)*** 3rd event: 5/26 (19%)***	1.12%^*	0.63%§
Recent infection + Recent allergy	1st event: 20/195 (10%)*** 2nd event: 11/67 (16%)*** 3rd event: 3/26 (12%)***	1.01%^*	0.54%§
SLEDAI-2K>4 + Recent Infection + Recent allergy	1st event: 7/202 (3%)*** 2nd event: 4/67 (6%)*** 3rd event: 2/26(8%)***	0.51%^**	0.11%§

Abbreviations/symbols. *: p>0.05; **: p<0.010; and ***: p<0.001 by binomial probability test. ^: calculated as the mean rate of each event per single patient per visit; § calculated by multiplying each isolated probability observed prospectively.

Consistently, at prospective analysis, LLDAS was inversely associated with a recent infection (χ^2=8.234; p=0.005; RR=0.67, 95% CI 0.51-0.88) or allergy (χ^2=20.912;

p<0.001; RR=0.16, 95% CI 0.06-0.39). Patients with a recent history of allergy were more likely to also report a history of a recent infection (χ^2=15.509; p<0.001; RR=4.08, 95% CI 1.92-8.67; **Figure 5**). Patients reporting a recent history of allergy were younger (median age 35 (24-46) years) than patients with no recent allergy (median age 43 (34-53); p=0.008) and had higher SLEDAI-2K scores (4 (3.5-11) vs 3 (2-4); p<0.001). Twenty-six out of 28 allergic events (93%) occurred in patients with a known history of allergy.

Figure 5: associations among SLE activity, allergic reactions and infections



Bar charts showing the relative frequencies of recent infections and allergic events among patients in remission vs with active disease according to the achievement of lupus low disease activity state (LLDAS) criteria (panel A, B). Panel C shows the relative frequency of allergic events among patients also reporting vs not reporting a recent infection. Abbreviations/symbols: **: p<0.01; ***: p<0.001.

Genetic studies

Frequency of HLA-DRB1 genotypes among patients and controls and in the general population

HLA-DRB1 genotype data were obtained from 185 patients with SLE, 80 patients with TAK and 86 healthy subjects. Population data from relatively large sample sizes were only available for low-resolution HLA-DRB1 genotyping. At low resolution, HLA-DRB1*03 was more prevalent in patients with SLE and HLA-DRB1*07 in patients with TAK than expected in the Italian general population (**Table 5**).

Table 5: low-resolution HLA-DRB1 allele frequencies

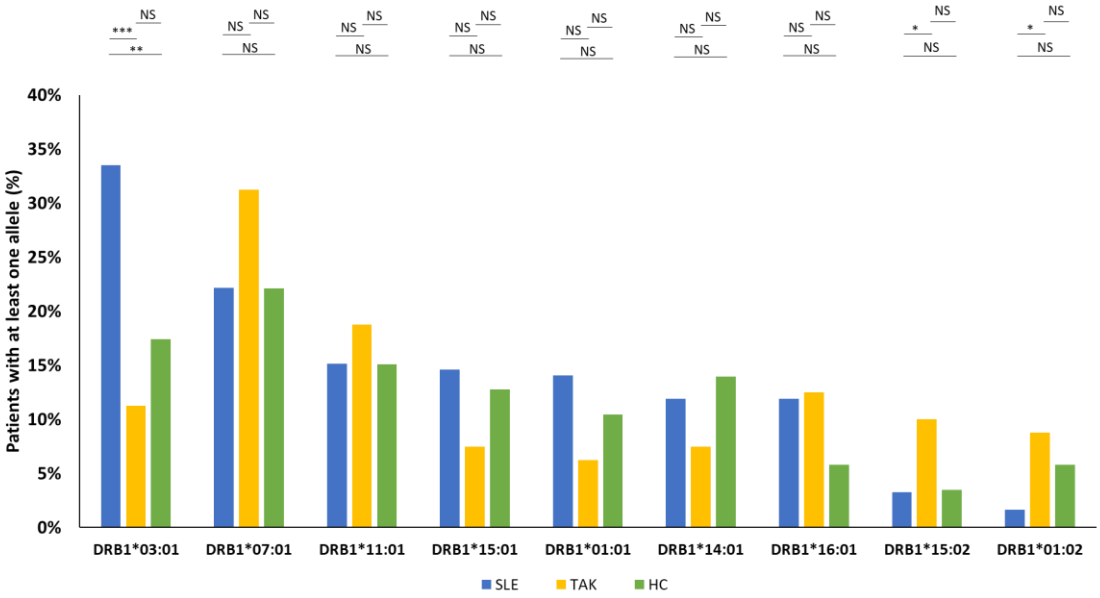
	SLE		TAK		HC		Italian General Population*	
	%	Sample size	%	Sample size	%	Sample size	%	Sample size
DRB1*03	34%	185	11%	80	17%	86	20%	159,311
DRB1*11	29%	185	40%	80	34%	86	48%	159,311
DRB1*07	22%	185	31%	80	22%	86	25%	159,311
DRB1*13	21%	185	24%	80	26%	86	31%	159,311
DRB1*15	18%	185	18%	80	16%	86	26%	159,311
DRB1*01	18%	185	15%	80	19%	86	18%	159,311
DRB1*04	15%	185	20%	80	24%	86	16%	159,311
DRB1*14	13%	185	9%	80	14%	86	10%	4,575
DRB1*16	13%	185	14%	80	7%	86	9%	4,575
DRB1*08	5%	185	5%	80	2%	86	6%	159,311
DRB1*10	3%	185	10%	80	3%	86	3%	159,311

*Data were retrieved from the Allele Frequency Net - http://www.allelefrequencies.net - (Gonzalez-Galarza, McCabe et al., 2019), based on data from multiple studies (De Re, Caggiari et al., 2010, Rendine, Borelli et al., 1998).

High-resolution data are shown in **Figure 6** and in **Appendix 2**. Consistent with low-resolution data, HLA-DRB1*03:01 was significantly more frequent in patients with SLE than in HC (χ^2=7.455; p=0.006) or in patients with TAK (χ^2=14.114; p<0.001). HLA-DRB1*15:01 and 01:01 were also more frequent in patients with SLE than in controls. HLA-DRB1*07:01 and 11:01 were more frequent in patient with SLE and with TAK

compared to HC. HLA-DRB1*15:02 and 01:02 were significantly more frequent in patients with TAK than in SLE (χ^2=5.095; p=0.035 and χ^2=7.815; p=0.010, respectively).

Figure 6: high-resolution HLA-DRB1 allele frequencies



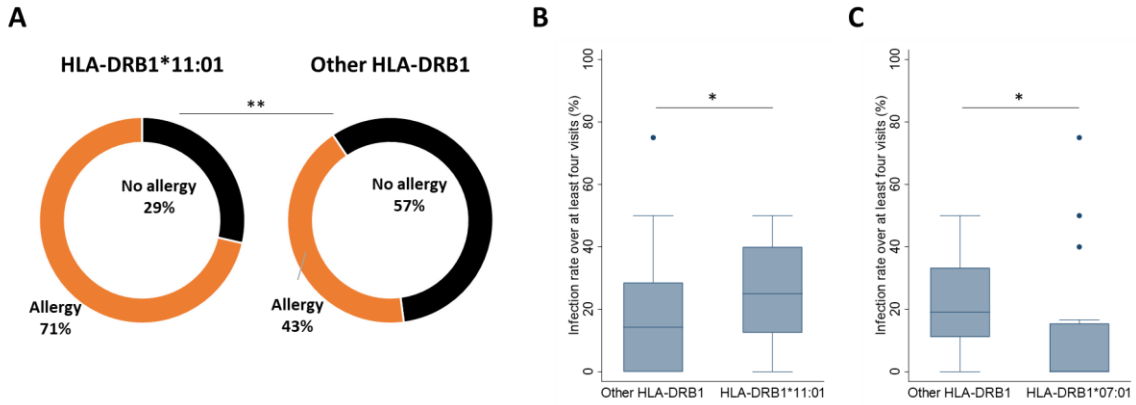
Bar chart showing the frequency of selected HLA-DRB1 genotypes in patients with SLE (blue), patients with TAK (yellow) and HC (green). Abbreviations/symbols: NS: non-significant; *: p<0.05; **: p<0.01; ***: p<0.001.

Genotype-phenotype associations

There was no specific association among autoimmune manifestations of SLE and HLA alleles. Patients with at least one HLA-DRB1*11:01 allele had more frequently a history of allergy (71%) than patients with other HLA-DRB1 variants (43%, χ^2=7.886; p=0.007). Among patients with data from at least four follow up visits in the frame of prospective clinical analyses (N=70), those with HLA-DRB1*11:01 reported higher rates of infections (25% of the visits, IQR 12.5-40%) compared to patients with other HLA profiles (14%, IQR 0-29%; p=0.044). Patients with at least one copy of the HLA-DRB1*07:01 reported lower rates of infections (<1%, 0-15% vs 19%, 11-33%; p=0.011; **Figure 7**). Of 160 genotyped patients who were monitored for SARS-CoV-2 infection, nine (5.6%) had symptomatic COVID-19 in 2020. Their clinical features are reported in **Appendix 3**. None of them carried a HLA-DRB1*07:01 allele (p=0.206). The frequency of HLA-DRB1*03:01 and of HLA-DRB1*11:01 alleles did not differ significantly among patients who had (22% both alleles) or did not have COVID-19 (34% and 16%,

respectively). Patients with HLA-DRB1*15:01 were significantly more frequent among COVID-19 cases (4/9, 44%) than among subjects without COVID-19 (19/151, 13%; p=0.025).

Figure 7: associations among HLA genotypes, allergic and infectious manifestations



This figure shows the associations among HLA genotypes, allergic manifestations and infectious events in patients with SLE. Patients with SLE who had at least one copy of the HLA-DRB1*11:01 allele had more frequently a history of allergy compared to patients with SLE with a different HLA profile (panel A). When observed prospectively over the course of at least four visits, patients with SLE with at least one HLA-DRB1*11:01 allele showed higher rates of infections (panel B), while patients with HLA-DRB1*07:01 alleles showed lower infection rates (panel C) compared to patients with other genotypes. Abbreviations/symbols: NS: non-significant; *: p<0.05; **: p<0.01; ***: p<0.001.

Peptide epitopes

Based on the results above, on literature review and on the use of an HLA binding prediction software (see Methods) a list of potential candidate autoantigens was generated and eventually shortened to a narrower list of potential peptide epitopes of interest (**Appendix 4 and 5**). The final list of peptides is reported in **Table 6**. Regarding autoantigens of interest, histone H3 and H4-derived peptides were preferred over peptides derived from Smith antigen, β2GPI, U1-RNP or other histone proteins due to the high prevalence of anti-nuclear immunity in the study population, existing evidence of CD4+ T-cell immunogenicity of at least one epitope in the literature (Lu et al., 1999) and high likelihood of binding to the study HLA-DRB1 molecules according to the Immune Epitope Database (IEDB) and the binding parameters requested by the MHC-tetramer manufacturer. A penicilloylated albumin peptide was selected as a marker of allergenicity due to the high-prevalence of beta-lactam allergy in the study population and the role of

penicilloylated albumin as the major antigenic determinant in beta-lactam hypersensitivity (Li, Yeung et al., 2020, Mirakian, Leech et al., 2015). Evidence of efficient binding to both HLA-DRB1 molecules and of CD4+ T cell immunogenicity further supported the choice of the penicilloylated albumin sequence reported in **Table 6** (Azoury et al., 2018). EBV latent-cycle sequences (EBNA1 and EBNA2) were selected as markers of the response to infectious agents, due to the high prevalence of EBV infection in the general population (Leogrande & Jirillo, 1993, Pordeus et al., 2008), a more relevant role of EBV over other pathogens in the pathogenesis of SLE (see also the Introduction) and to evidence of CD4+ T cell response and HLA binding to epitopes from the selected antigens (Draborg et al., 2014, Draborg, Jorgensen et al., 2012, Draborg et al., 2018, Draborg, Sandhu et al., 2016, Long, Chagoury et al., 2013, Meckiff, Ladell et al., 2019).

Table 6: synthetic peptide sequences employed for T-cell studies

Peptide #	Antigen	HLA restriction	Peptide sequence (aminoacid number)	Reference
1	Penicilloylated albumin	DRB1*03:01 DRB1*11:01	PELLFFAK*RYKAAFT	(Azoury et al., 2018)
2	Histone H3	DRB1*11:01	LPFQRLMREIAQD (66-78)	ND
3	Histone H4	DRB1*03:01	GLIYEETRGVLKVFL (49-63)	(Lu et al., 1999)
4	EBNA1	DRB1*11:01	HIFAEVLKDAIKDL (569-582)	ND
5	EBNA 2	DRB1*03:01	PAQPPPGVINDQQLHHLPSG (301-320)	(Long et al., 2013, Meckiff et al., 2019)

*: denotes penicilloylation of the lysine residue. Abbreviations. ND: no data

T cell phenotype

General features

Thirty-two patients with SLE, 11 patients with TAK and 10 HC (one of whom tested for both HLA-DRB1*03:01 and HLA-DRB1*11:01-related epitopes) with compatible HLA-DRB1 had sufficient cell material and were comparable in terms of sex distribution and age to be included in T-cell studies. Demographics are reported in **Table 7** and **Appendix 6**. Additional details on SLE-specific features, including laboratory test results at time of blood sampling and clinimetrics, are reported in **Table 8**. Five patients with SLE were tested during remission and active disease, as an exploratory analysis on longitudinal variations of T cell parameters over time. All these five patients were taking

mycophenolate mofetil to maintain the remission phase, whereas they were on azathioprine (n=3) or no immunosuppression (n=2) during the active phase. The median (IQR) prednisone dose in this subset of patients was 5 (5-5) mg/day during the active phase and 5 (0-5) mg/day during the remission phase.

Table 7: subjects included in T-cell studies

	SLE (N=32)	TAK (N=11)	HC (N=10*)
Females: N (%)	28 (88)	10 (91)	9 (90)
Age (y): median (IQR)	41 (34-51)	44 (36-47)	41 (34-51)
HLA-DRB1*03:01: N(%)	15 (47)	4 (36)	6 (60)
HLA-DRB1*11:01: N(%)	19 (59)	7 (64)	5 (50)
Disease duration (y): median (IQR)	17 (9-23)	10 (7-15)	NA
Prednisone dose (mg/day): median (IQR)	1 (0-4)	5 (3-5)	0

* One of the ten HC had both HLA-DRB1 alleles of interest and was therefore tested for T responses to both HLA-DRB1*03:01- and HLA-DRB1*11:01-bound peptides.

Table 8: SLE-specific clinical features of patients included in T-cell studies

Parameter	Value	
Disease activity indices		
LLDAS: N(%)	23 (72)	
SLEDAI-2K: median (IQR)	2 (2-4)	
Total BILAG score: median (IQR)	1 (0-9)	
PGA: median (IQR)	1 (0-1)	
Patient NRS: median (IQR)	7 (7-8)	
Chronic damage (SDI): median (IQR)	1 (0-2)	
Laboratory features	Value	Reference range
Haemoglobin (g/dl): median (IQR)	13 (12-15)	F: 12-16 M: 14-18
Platelets x 10^3/microlitre: median (IQR)	255 (215-324)	130-400
WBC (cells/microlitre): median((IQR)	5,900 (4,480-7,425)	4,800-10,800
Neutrophils (%): median((IQR)	60 (54-66)	40-75
Lymphocytes (%): median((IQR)	29 (23-33)	20-50
Monocytes (%): median((IQR)	8 (7-9)	2-15
Eosinophils (%): median((IQR)	2 (1-3)	1-6
Basophils (%): median((IQR)	1 (0-1)	0-2
ESR (mm/h): median((IQR)	11 (6-32)	1-20
CRP (g/l): median((IQR)	1 (0-4)	<6
Serum creatinine (mg/dl): median((IQR)	0.8 (0.7-1.0)	0.5-1.1
Complement C3 (mg/l): median((IQR)	0.95 (0.76-1.07)	0.90-1.80
Complement C4 (mg/l): median((IQR)	0.19 (0.13-0.21)	0.10-0.40

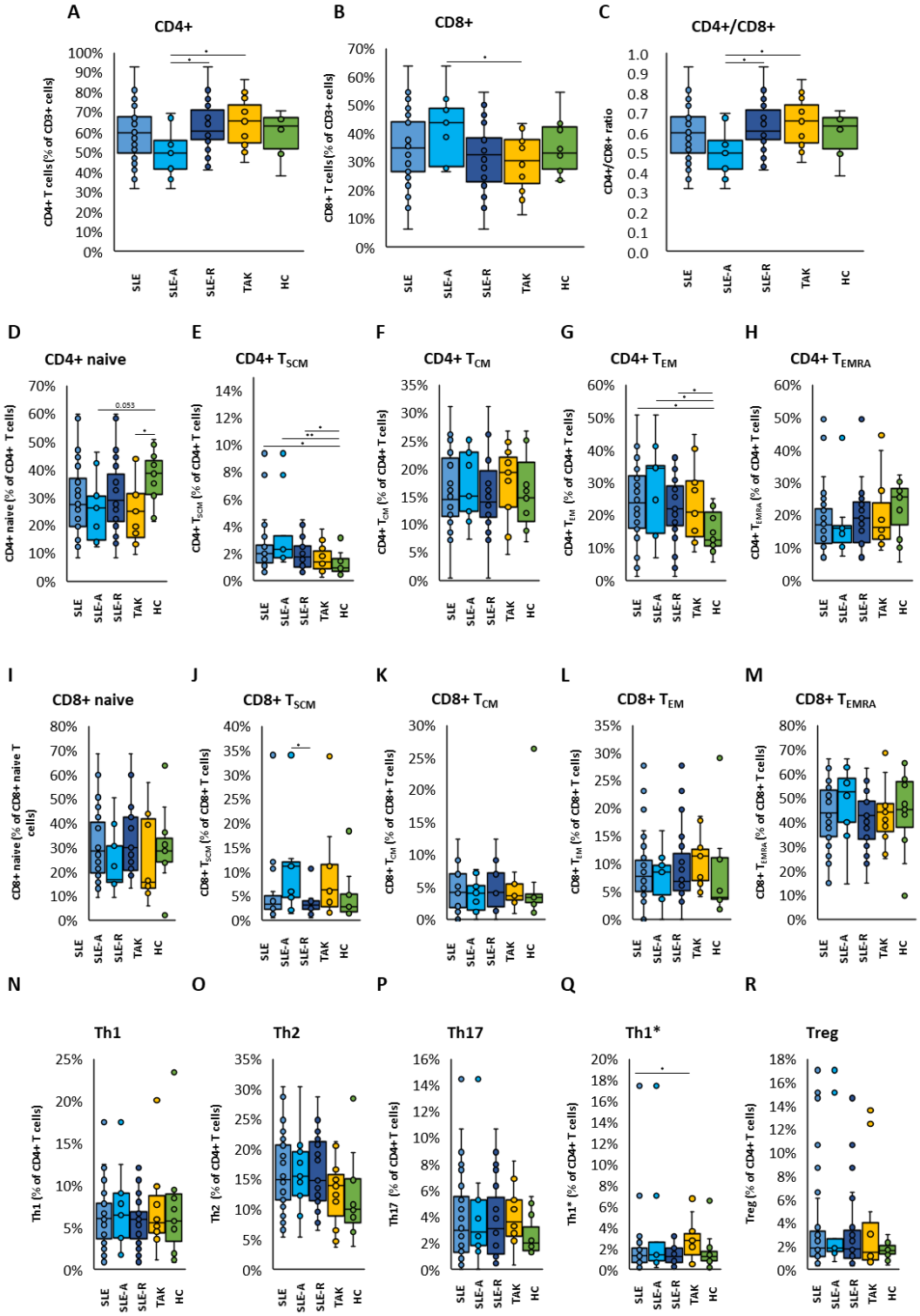
Positive aDNA: N (%)	18 (56)	Negative
aDNA titre: N(%)		
borderline	2 (11)	Negative
low	4 (22)	Negative
moderate	8 (44)	Negative
high	2 (11)	Negative
very high	2 (11)	Negative
Treatment features: N (%)	Current	Ever
Immunosuppressants	18 (56)	30 (81)
Cyclophosphamide	0 (0)	8 (25)
MMF	12 (38)	15 (47)
MTX	3 (9)	10 (31)
AZA	2 (6)	14 (44)
Cyclosporine A	0	5 (16)
RTX	1 (3)	4 (13)
Immunomodulants	28 (88)	31 (97)
HCQ	28 (88)	31 (97)
BEL	1 (3)	2 (6)

At basic flow cytometry analysis, there was no significant difference among patients with SLE and controls in terms of circulating CD4+ or CD8+ T lymphocytes. Patients with active SLE (LLDAS=0) had lower CD4+ counts (49% of total CD3+, IQR=42-56%) compared to patients with SLE in remission (61%, IQR=56-73%; p=0.020) and to patients with TAK (65%, IQR=53-67%; p=0.038). Patients with active SLE had higher CD8+ counts (44% of total CD3+, IQR=28-49%) than patients with TAK 30%, IQR=20-39%; p=0.044). Accordingly, patients with active SLE had lower CD4/CD8 ratios than patients with SLE in remission and patients with TAK.

Patients with SLE had significantly more circulating T_{SCM} (2.00%, IQR=1.25-2.62%) compared to HC (0.91%, IQR=0.67-1.77%; p=0.012). Circulating CD4+ T_{EM} were increased in patients with SLE (24%, IQR=16-33%) as compared to HC (13%, IQR=10-23%; p=0.014). T_{SCM} and T_{EM} populations were expanded in patients with active SLE (2.30%, IQR=1.70-3.27% and 34%, IQR 14-35%; p=0.007 and p=0.030, respectively). Conversely, naïve CD4+ cells were more represented among HC (38%, IQR=31-45%) than among patients with TAK (25%, IQR=14-32%; p=0.024) and active SLE (26%, IQR=15-31%; p=0.053). The difference between the proportion of CD8+ T_{SCM} among patients with active (11%, IQR=5-12%) vs non active SLE (3%, IQR=2-4%; p=0.014) was also statistically significant.

In terms of T cell polarisation, there was no statistically significant difference among patients with SLE with or without active disease and HC, although Th2 cells were more represented within the CD4+ repertoire in patients with SLE than in controls. Among patients with SLE, patients on immunosuppressants had lower Th1 (4.23% CD4+ cells, IQR=2.21-6.44%) and Th2 cells (12.30% CD4+ cells, IQR=9.61-15.13%) than patients without immunosuppressants (7.20% Th1/CD4+ cells, IQR=5.31-10.61%, p=0.028; 20.13% Th2/CD4+ cells, IQR=17.74-22.90%, p=0.002). Th1* cells were more expanded in patients with TAK (2.73%, IQR=0.65-3.61%) compared to patients with SLE (1.27%, IQR=0.67-2.06%, p=0.048; **Figure 8**).

Figure 8: T-cell memory and polarisation phenotype in patients and controls



Boxplots showing the differential distribution of T cells in patients with SLE (blue) with active disease (bright blue, SLE-A) or in remission (dark blue, SLE-R), patients with Takayasu's arteritis (TAK, yellow) and healthy controls (HC, green), as assessed by flow cytometry. Panel A-B depict the frequency of CD4+ and CD8+ T lymphocytes, whereas the ratio between CD4+ and CD8+ cells is shown in panel C. Panel D-H show the frequency of CD4+ T cells according

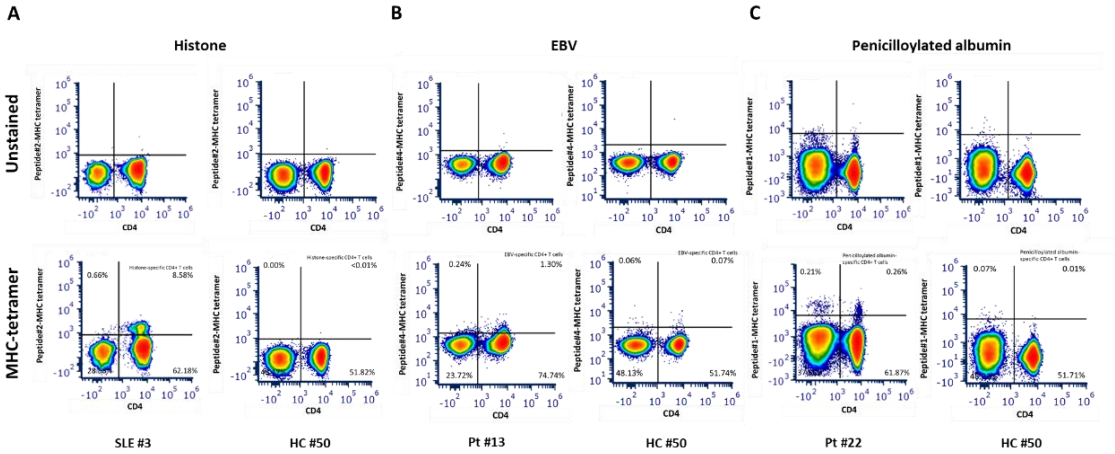
to their memory/differentiation phenotype. Panel I-M show the frequency of CD8+ T cells according to their memory/differentiation phenotype. Panel N-R show the frequency of CD4+ T cells according to their polarisation.

Abbreviations. T_{SCM}: stem-cell memory T cells, T_{CM}: central memory T cells, T_{EM}: effector memory T cells, T_{EMRA}: terminally differentiated T cells. *: p<0.05; **:p<0.01. For further details on the gating strategy see **Table 14** and **Table 15.**

Antigen-specific T-cell phenotype

Antigen-specific CD4+ events were detected at low frequencies in patients and controls (**Figure 9**).

Figure 9: antigen-specific T cells



Representative density plots elaborated on flow cytometry data and showing the frequency of histone- (A), EBV- (B), and penicilloylated albumin-specific (C) CD4+ T cells in three patients with SLE (left) and one HC (right). The upper plots shows the control signal obtained without tetramer staining.

Histone-specific responses in patients and controls

Histone-specific CD4+ T cells above the diagnostic threshold for positive ADNA in patients' history (**Appendix 7**) were more frequent in patients with SLE (23/32, 72%) than in HC (0/(10+1); p<0.001) and TAK (3/11, 27%; p<0.014). Two of the three patients with TAK with histone-specific CD4+ T cells (patient #36 and patient #39) had positive ANA in their clinical history.

Quantitatively, the fraction of absolute total histone-specific CD4+ T cells over CD3+ cells was higher in SLE (4.03‰, IQR=3.54-4.26‰) than in HC (0.09‰, IQR=0-0.25; p<0.001) and TAK (0.51‰, 0-2.13‰; p=0.008). The corresponding fractions of CD4+

cells were 6.37‰ (6.23-8.84) in SLE, 0.14‰ (0-0.41) in HC (p<0.001 vs SLE) and 0.76‰ (0-2.46) in TAK (p=0.003 vs SLE).

When compared to HC, patients with SLE showed a harmonic expansion of histone-specific CD4+ T cells at different stages of differentiation and with distinct polarisations. Specifically, histone-specific CD4+ naïve, T_{SCM}, T_{CM} and T_{EM} cells and histone-specific Th2, Th17 and Treg (along with similar trends for Th1 and Th1*) cells were all expanded in patients with SLE compared to HC. The same findings were obtained by either considering the fraction of each subpopulation over the total CD3+ or CD4+ cells. Within the histone-positive compartment, T_{CM} were particularly expanded in patients with SLE. Similar findings were obtained in comparison with patients with TAK, who showed lower levels of circulating histone-specific CD4+ naïve, T_{CM} and T_{EM} cells and lower histone-specific Th2, Th17, Th1 and Treg cells compared to patients with SLE (**Table 9**).

Table 9: histone-specific CD4+ T cell subpopulations in patients and controls

Antigen-specific CD4+ T cells	Median (IQR) n/10^5 CD3+ cells			Median (IQR) n/10^5 of CD4+ cells			Median (IQR) % of Tetramer+ cells		
	SLE	TAK	HC	SLE	TAK	HC	SLE	TAK	HC
By memory phenotype									
Naïve	8.8 (0.8-102.88)§§^	0 (0-1.52)	0 (0-1.99)	15.68 (1.81-143.33)§§^	0 (0-1.76)	0 (0-3.23)	28 (10-44)	0 (0-19)	13 (0-33)
T_{SCM}	3.38 (0-44.92)§§	0 (0-1.03)	0 (0-0)	4.7 (0-69.71)§§	0 (0-1.48)	0 (0-0)	6 (0-15)	2 (0-15)	0 (0-4)
T_{CM}	15.01 (3.27-66.96)§§§^	1.01 (0-5.57)§	0 (0-0.62)	25.23 (5.54-110.68)§§§^	1.51 (0-7.27)	0 (0-1.02)	22 (12-35)§	27 (15-40)	0 (0-9)
T_{EM}	8.33 (2.1-24.84)§§§^^	0 (0-0.88)	0 (0-0.49)	13.33 (4.36-37.92)§§§^^	0 (0-1.29)	0 (0-0.73)	10 (5-29)	5 (0-13)	0 (0-9)
T_{EMRA}	1.98 (0-12.51)	0 (0-0.51)	0 (0-0.94)	3.09 (0-23.37)	0 (0-0.76)	0 (0-1.53)	3 (0-12)	0 (0-13)	9 (0-23)
By polarisation phenotype									
Th1	0 (0-9.51)	0 (0-0)	0 (0-0)	0 (0-12.29)	0 (0-0)	0 (0-0)	0 (0-5)	0 (0-1)	0 (0-1)
Th2	8.28 (1.17-26.1)§§^^	0 (0-0.92)	0 (0-1.21)	11.87 (2.3-36.51)§§^^	0 (0-1.06)	0 (0-2.02)	13 (3-32)	0 (0-19)	1 (0-22)
Th17	0 (0-3.39)§^	0 (0-0)	0 (0-0)	0 (0-5.47)§^	0 (0-0)	0 (0-0)	0 (0-5)	0 (0-0)	0 (0-0)
Th1*	0 (0-2.59)^	0 (0-0)	0 (0-0)	0 (0-4.78)^	0 (0-0)	0 (0-0)	0 (0-2)	0 (0-0)	0 (0-0)
Treg	1.91 (0-43.39)^	0 (0-0.46)	0 (0-0.3)	2.76 (0-54.46)§^	0 (0-0.53)	0 (0-0.5)	8 (0-24)	0 (0-5)	4 (0-27)

^:p<0.05, ^^:p<0.01 vs TAK; §: p<0.05, §§: p<0.01, §§§: p<0.001 vs HC

EBV-specific responses in patients and controls

Eight out of ten subjects with established positive EBV status had detectable EBV-specific CD4+ T cell events. With EBV-specific CD4+ T cell count threshold set on the median count in this subset, EBV-specific CD4+ T cell-positive subjects tended also to be more frequent in patients with SLE (18/32, 56%) than in HC (3/(10+1), 27%; p=0.162) and TAK (4/11, 36%, p=0.310).

Patients with SLE had a higher proportion of EBV-specific absolute total CD4+ T cell counts over total CD3+ cells (6.38‰, IQR=0.44-20.24‰) and CD4+ cells (12.25‰, IQR=0.78-28.27) than HC (1.01‰ of CD3+ cells, IQR=0.00-2.86‰; p=0.039; and 1.61‰ of CD4+ cells, IQR=0.00-5.85‰; p=0.041).

The fraction of EBV-specific CD4+ T_{SCM} cells over CD3+ cell count was higher in patients with SLE than in HC, whereas EBV-specific Th1* cells were relatively more frequent in SLE than in TAK. There were no substantial differences among TAK and HC (**Table 10**).

Table 10: EBV-specific CD4+ T cell subpopulations in patients and controls

Antigen-specific CD4+ T cells	Median (IQR) n/10^5 CD3+ cells			Median (IQR) n/10^5 of CD4+ cells			Median (IQR) % of Tetramer+ cells		
	SLE	TAK	HC	SLE	TAK	HC	SLE	TAK	HC
By memory phenotype									
Naïve	31.73 (0-124.53)	0 (0-40.01)	3.9 (0-23.18)	63.26 (0-200.97)	0 (0-60.67)	5.87 (0-52.04)	38 (10-58)	32 (0-48)	36 (28-49)
T_{SCM}	14.41 (0-32.56)§	1.01 (0-17.32)	0.98 (0-3.43)	24.29 (0-54.07)	1.51 (0-29.08)	1.47 (0-6.84)	11 (3-27)	11 (2-19)	6 (3-9)
T_{CM}	4.19 (0-56.31)	0 (0-12.57)	2.93 (0-12.47)	8.09 (0-97.65)	0 (0-16.94)	4.4 (0-29.47)	11 (1-23)	7 (0-18)	15 (12-21)
TEM	4.07 (0-41.52)	1.01 (0-34.4)	2.28 (0-9.95)	9.02 (0-74.79)	1.51 (0-58.63)	3.3 (0-21.27)	7 (0-13)	13 (7-28)	14 (8-20)
T_{EMRA}	9.05 (0-27.98)	1.01 (0-10.84)	2.93 (0-12.83)	15.34 (0-43.43)	1.51 (0-19.16)	4.4 (0-22.01)	6 (2-20)	10 (7-18)	19 (13-27)
By polarisation phenotype									
Th1	0 (0-0)	0 (0-0.46)	0 (0-3.73)	0 (0-0)	0 (0-0.53)	0 (0-5.85)	0 (0-0)	0 (0-1)	0 (0-2)
Th2	0 (0-2.53)	0 (0-0.92)	0 (0-4.69)	0 (0-3.86)	0 (0-1.06)	0 (0-8.18)	0 (0-4)	0 (0-3)	0 (0-2)
Th17	0 (0-2.11)	0 (0-0.3)	0 (0-3.37)	0 (0-3.3)	0 (0-0.49)	0 (0-6.03)	0 (0-1)	0 (0-0)	0 (0-1)
Th1*	0 (0-4.59)^	0 (0-0)	0 (0-12.24)	0 (0-6.75)^	0 (0-0)	0 (0-19.36)	0 (0-7)	0 (0-0)	0 (0-7)
Treg	3.81 (0-27.66)	0 (0-4.62)	0 (0-87.7)	5.09 (0-43.65)	0 (0-5.28)	0 (0-135.45)	9 (0-52)	0 (0-9)	0 (0-41)

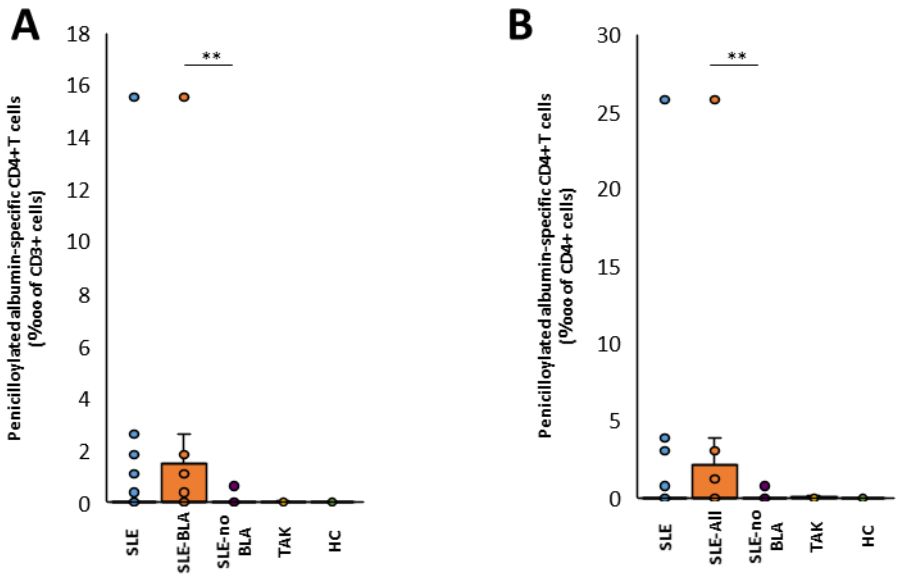
^:p<0.05, ^^:p<0.01 vs TAK; §: p<0.05, §§: p<0.01, §§§: p<0.001 vs HC

Penicilloylated albumin-specific responses in patients and controls

Penicilloylated albumin-specific CD4+ T cells above the diagnostic threshold for beta-lactam allergy (**Appendix 7**) were absent in patients with TAK and HC and detected in 5/11 (45%) patients with SLE with beta-lactam allergy and 1/21 (5%) patients with SLE without beta-lactam allergy (χ^2=7.846; p=0.011). This latter patient (patient #29) had, however, a history of allergy to tetracyclines, which might have constituted a confounder (Hamilton & Guarascio, 2019). Patients with SLE and beta-lactam allergy had higher levels of absolute total anti-penicilloylated albumin-specific CD4+ T cells over the total count of CD3+ and CD4+ cells than patients with SLE and no beta-lactam allergy (p=0.005 for both variables; **Figure 10**). Patients with SLE and beta-lactam allergy had

lower EBV-specific CD4+ T_{EM} cells with reference to total CD4+ cells (0‰, IQR=0-1.19‰) than patients without allergy history (2.04‰, IQR=0-8.66 ‰; p=0.038). No other significant differences were found in terms of histone or EBV-specific CD4+ T cells between these groups. Penicilloylated albumin-specific CD4+ T cells were expanded at various stages of differentiations and with prominent expansion of the extreme (naïve and T_{EMRA}) subpopulations. In terms of polarisation, Th2 and Treg cells recognising penicilloylated peptides were more significantly expanded in patients with SLE and beta-lactam allergy (**Table 11**).

Figure 10: total penicilloylated albumin-specific T cells



This figure shows the proportion of antigen-specific CD4+ T cells recognising penicilloylated-albumin peptides over the total CD3+ (A) and CD4+ counts (B) in patients with SLE, TAK and HC. Abbreviations. SLE-BLA: SLE and beta-lactam allergy; SLE no-BLA: SLE without beta-lactam allergy. **:p<0.01.

Table 11: penicilloylated albumin-specific CD4+ T cells in patients with SLE

Antigen-specific CD4+ T cells	Median (IQR) n/10^5 CD3+ cells		Median (IQR) n/10^5 of CD4+ cells		Median (IQR) % of Tetramer+ cells	
	SLE - allergy to beta-lactams	SLE - no allergy to beta-lactams	SLE - allergy to beta-lactams	SLE - no allergy to beta-lactams	SLE - allergy to beta-lactams	SLE - no allergy to beta-lactams
By memory phenotype						
Naive	0 (0-18.2)^^	0 (0-0)	0 (0-24.94)^^	0 (0-0)	50 (33-71)	35 (35-35)
T_{SCM}	0 (0-0.48)^	0 (0-0)	0 (0-0.63)^	0 (0-0)	1 (0-5)	0 (0-0)
T_{CM}	0 (0-1.44)^	0 (0-0)	0 (0-1.89)^	0 (0-0)	2 (0-3)	0 (0-0)
T_{EM}	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
T_{EMRA}	0 (0-24.67)^^	0 (0-0)	0 (0-39.62)^^	0 (0-0)	29 (22-45)	65 (65-65)
By polarisation phenotype						
Th1	0 (0-0)^	0 (0-0)	0 (0-0)^	0 (0-0)	0 (0-6)	0 (0-0)
Th2	0 (0-1.29)^^	0 (0-0)	0 (0-1.52)^^	0 (0-0)	8 (6-13)	0 (0-0)
Th17	0 (0-0.41)^	0 (0-0)	0 (0-0.51)^	0 (0-0)	5 (0-6)	0 (0-0)
Th1*	0 (0-1.64)	0 (0-0)	0 (0-2.02)	0 (0-0)	25 (0-27)	100 (100-100)
Treg	0 (0-5.53)^^	0 (0-0)	0 (0-7.6)^^	0 (0-0)	28 (17-69)	0 (0-0)

^:$p<0.05$, ^^:$p<0.01$.

Comparison of antigen-specific T cell profiles in patients with SLE

In patients with SLE, the fraction of histone-specific CD4+ T cells over total CD3+ and CD4+ cells was correlated to the respective fractions of EBV-specific CD4+ T cells ($\rho=0.464$; $p=0.008$ and $\rho=0.412$; $p=0.019$, respectively). Patients with beta-lactam allergy also showed a positive correlation among the fraction of penicillin-specific CD4+ T cells over the whole CD3+ and CD4+ population and the fraction of EBV-specific CD4+ T cells over the total CD4+ count ($\rho=0.612$; $p=0.026$ for both variables). This correlation was possibly more evident in patients with beta-lactam allergy and active disease ($\rho>0.999$; $p=0.001$; $n=4$). In patients with SLE the fraction of total histone-specific CD4+ T cells over CD3+ and CD4+ cells were also correlated to platelet count at time of clinical evaluation ($\rho=0.388$; $p=0.028$ and $\rho=0.360$; $p=0.043$, respectively). There was no correlation among ADNA titres at time of sampling and total histone-specific T cells. ADNA titres at time of sampling also showed no correlation with the majority of histone-specific CD4+ T cell subpopulation counts and were only slightly inversely correlated with the proportion of histone-specific Th1* cells over total CD3+ and CD4+ cells ($\rho=-0.382$; $p=0.034$ and $\rho=-0.387$; $p=0.031$, respectively).

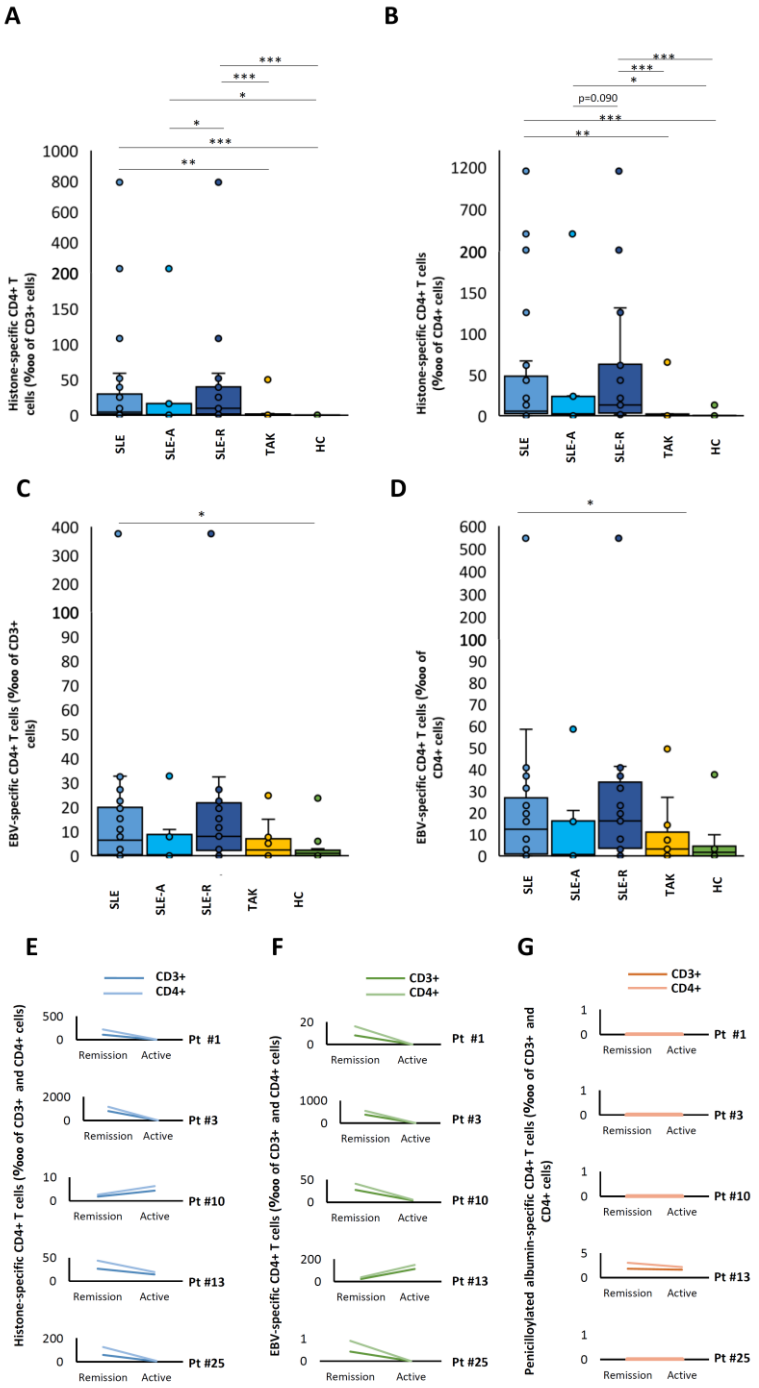
Impact of SLE activity on antigen-specific T cells

There were no substantial differences in total antigen-specific T cells and in antigen-specific T cell subpopulations when patients with SLE were stratified by disease manifestations. In terms of disease activity, patients with active disease had lower total histone-specific CD4+ T cell counts (0.76‰ of CD3+ cells, IQR=0.43-16.22‰ and 2.43‰ of CD4+ T cells, IQR=0.97-23.59‰) than patients fitting the criteria for LLDAS (9.52‰ of CD3+ cells, IQR=1.75-39.87‰; p=0.047; and 13.01‰ of CD4+ T cells, IQR=3.13-63.74‰; p=0.089). Consistently, patients tested in both conditions had higher total histone-specific CD4+ T cell counts when in remission than during active disease (median Δ= -55‰ of CD3+ and median Δ=-117‰ of CD4+ during active disease; p=0.089). Total EBV-specific T cells tended to decrease during disease activity. Patient #13 who was allergic to beta-lactams and was tested during remission and activity, showed a decrease in total penicilloylated albumin-specific CD4+ T cells during disease activity (**Figure 11**).

When the comparison among patients with active disease and remission was extended to antigen-specific subpopulations, histone-specific and EBV-specific CD4+ T cell subpopulations were found generally decreased with regard to total CD3+, CD4+ and tetramer+ cells. Histone-specific and EBV-specific CD4+ T_{EM} cells were more prominently reduced in patients with active disease. A descending trend of histone-specific CD4+ T_{EM} cells was also observed in patients tested during remission and active disease (median Δ= -11‰ of CD3+ and median Δ=-22‰ of CD4+ during active disease; p=0.080), who also showed a relative increase in histone-specific T_{CM} (median Δ=13% of tetramer+ cells; p=0.043) and a decrease in EBV-specific CD4+ T_{SCM} (median Δ=-25% of tetramer+ cells; p=0.043). Histone-specific Th2 and Treg (and less strongly Th1 and Th1*) cells were all significantly reduced in patients with active disease. An activity-related reduction in histone-specific Th2 cells (median Δ= -7‰ of CD3+ and median Δ=-14‰ of CD4+ compared to remission; p=0.043), along with similar trends in the other Th subpopulations, were consistently observed in patients with SLE tested under different disease activity states. Furthermore, histone specific CD4+ Treg cells were inversely correlated with SLEDAI-2K (ρ=-0.437; p=0.012) and showed a possible hyperbolic decrease with increasing SLEDAI-2K values (**Appendix 8**). EBV-specific CD4+ T cell reduction was more prominent in Th17, Th1* and Treg subpopulations

(**Table 12**). In patients tested twice, ADNA titres did not change significantly from remission to active disease and were not correlated to changes in histone-specific T cell subpopulations.

Figure 11: total antigen-specific T cells by disease activity



This figure shows variations of total antigen-specific CD4+ T cells with disease activity in patients with SLE and provides comparative information from control groups. Boxplots A-D report the frequency of histone-specific (panel A-B) and EBV-specific (panel C-D) CD4+ T cells over the total CD3+ and CD4+ counts in patients with SLE, TAK and HC. Panel E-G show the variations in the proportion of histone specific (E, blue), EBV-specific (F, green) and penicilloylated albumin-specific (G, orange) CD4+ T cells in five patients with SLE with repeat samples during remission and active disease. Abbreviations. SLE-A: active SLE; SLE-R: SLE in remission. *: $p<0.05$; **:$p<0.01$; ***:$p<0.001$.

Table 12 antigen-specific CD4+ T cell subpopulations in patients with SLE by disease activity

Antigen-specific CD4+ T cells	Median (IQR) n/10^5 CD3+ cells		Median (IQR) n/10^5 of CD4+ cells		Median (IQR) % of Tetramer+ cells	
	SLE - active	SLE- remission	SLE - active	SLE- remission	SLE - active	SLE- remission
Histone-specific CD4+ T cells						
By memory phenotype						
Naïve	0 (0-50.87)	13.27 (3.51-146.34)	0 (0-73.09)	22.38 (6.12-219.51)	25 (0-36)	31 (12-49)
T_{SCM}	0 (0-11.56)	3.95 (0-51.05)	0 (0-16.61)	6.07 (0-86.26)	7 (0-13)	4 (0-15)
T_{CM}	1.45 (0-46.25)	19.74 (8.8-77.18)	2.94 (0-66.44)	28.49 (13.2-128.29)	23 (4-28)	20 (13-38)
T_{EM}	2.18 (0-7.25)^	11.44 (4.04-35.24)	6.02 (0-14.71)	21.16 (6.08-69.64)	33 (9-38)	9 (5-22)
T_{EMRA}	0 (0-4.35)	3.07 (0-25.83)	0 (0-8.83)	5.07 (0-48.57)	5 (0-20)	3 (0-11)
By polarisation phenotype						
Th1	0 (0-0)^^	4.72 (0-24.5)	0 (0-0)^^	5.11 (0-46.39)	0 (0-0)	2 (0-10)^
Th2	0 (0-3.33)^^	10.37 (5.15-69.39)	0 (0-6.27)^^	14.15 (9.71-129.84)	13 (0-18)	13 (6-34)
Th17	0 (0-0.83)	0.94 (0-4.77)	0 (0-1.57)	1.02 (0-7.54)	0 (0-4)	0 (0-4)
Th1*	0 (0-0)^^	1.71 (0-9)	0 (0-0)^^	2.75 (0-11.2)^^	0 (0-0)	0 (0-4)^
Treg	0 (0-0)^^^	9.49 (1.2-110.27)	0 (0-0)^^^	13.74 (1.86-178.97)^^^	0 (0-0)	13 (4-43)^^
EBV-specific CD4+ T cells						
By memory phenotype						
Naïve	0 (0-108.15)	35.5 (0-146.82)	0 (0-174.4)	71.74 (0-207.57)	33 (6-52)	38 (20-60)
T_{SCM}	0 (0-21.63)	18.52 (4.7-34.36)	0 (0-32.25)	36.53 (7.27-56.26)	19 (3-27)	11 (3-25)
T_{CM}	0 (0-10.82)	8.81 (0-69.86)	0 (0-16.12)	15.06 (0-106.74)	10 (1-21)	11 (1-23)
T_{EM}	0 (0-0)^	9.8 (0.64-47.71)	0 (0-0)^	14.89 (0.76-78.35)	0 (0-7)	8 (2-15)
T_{EMRA}	0 (0-13.06)	9.65 (0.49-28.56)	0 (0-26.48)	16.28 (0.81-43.6)	7 (1-23)	6 (3-18)
By polarisation phenotype						
Th1	0 (0-0)	0 (0-0.85)	0 (0-0)	0 (0-1.6)	0 (0-0)	0 (0-5)
Th2	0 (0-0)	0 (0-3.31)	0 (0-0)	0 (0-5.79)	0 (0-1)	0 (0-6)
Th17	0 (0-0)^	0 (0-2.55)	0 (0-0)^	0 (0-4.61)	0 (0-0)	0 (0-4)
Th1*	0 (0-0)^	2.59 (0-6.75)	0 (0-0)^	5.5 (0-10.77)	0 (0-0)	4 (0-12)
Treg	0 (0-0)^^	8.49 (0.86-37.69)	0 (0-0)^^	9.6 (1.17-59.66)	0 (0-0)	24 (5-71)

^:p<0.05, ^^:p<0.01, ^^^:p<0.001.

Associations among antigen-specific T cells and treatments in patients with SLE

In patients with SLE, there was no significant correlation among immunosuppressant or prednisone dose and antigen-specific T cell counts. Patients taking at least one immunosuppressant (n=18/32, **Table 8**) had higher naïve histone-specific CD4+ T cells (3.51‰ CD3+, IQR=0.31-26.46‰; 5.01‰ CD4+, IQR=0.72-32.61‰) than patients without immunosuppressants (0.33‰ CD3+, IQR=0-0.88‰, p=0.044; 0.50‰ CD4+, IQR=0.72-1.34‰, p=0.034). Histone-specific T_{EMRA} were also higher in patients on immunosuppressants (0.73‰ CD3+, IQR=0-6.66‰; 0.17‰ CD4+, IQR=0-8.89‰) than in patients on immunomodulant treatment only (0‰ CD3+, IQR=0-0.36‰, p=0.023; 0‰ CD4+, IQR=0-0.63‰, p=0.025). Within the histone-specific compartment, Th2 cells were more represented in patients without immunosuppressants

(33.64%, IQR=12.66-40.00%) than in patients on immunosuppressants (9.29%, IQR=0.95-14.81%; p=0.010). There were no significant variations in EBV-specific and penicilloylated albumin-specific T cell counts with taking at least immunosuppressant.

Regarding specific treatments, (**Table *8***), there was no significant difference in antigen-specific T cell counts when patients were stratified for current or past history of treatment with azathioprine, cyclosporine A, cyclophosphamide, rituximab and belimumab. Patients having ever been treated with methotrexate had higher total EBV-specific T cell counts over the total CD3+ T cell count (19.45‰, IQR=7.83-32.38‰) than the remainder patients (2.17‰, IQR=0-8.70‰, p=0.031). Patients with a history of methotrexate treatment also had higher EBV-specific T_{EMRA} cells (2.76‰ CD4+, IQR=0.97-5.41‰) than patients having received different treatments (0.05‰ CD4+, IQR=0-1.26‰; p=0.038).

Patients with a current (n=12, 9/12 in remission) and/or past history (n=15) of treatment with mycophenolate mofetil had higher levels of total histone-specific CD4+ T cells over CD3+ count (22.16‰, IQR=5.63-56.29‰ and 12.00‰, IQR=3.52-39.11‰, respectively) than patients having never received mycophenolate mofetil (1.91‰, IQR=0.99-14.76‰, p=0.037 and 1.74‰, IQR= 0.72-13.29, p=0.034, respectively). Patients having been treated with mycophenolate mofetil during their disease history also had a higher fraction of histone-specific CD4+ T cells over the total CD4+ T cell count (16.15‰, IQR=5.30-53.79‰) than the remainder patients (3.37‰, IQR=1.64-21-98‰, p=0.045). A current and/or past history of mycophenolate mofetil also associated with higher histone-specific CD4+ T_{EM}: 3.34‰ CD4+, IQR=1.12-16.69‰ in patients on mycophenolate at time of sampling vs 0.66‰, IQR=0.11-2.14‰ in patients treated with other drugs (p=0.015); 2.23‰ CD4+, IQR=0.76-6.31‰ in patients having ever been treated with mycophenolate mofetil vs 0.60‰, IQR=0-2.12‰ in the remainder patients with SLE (p=0.037). The fraction of histone-specific T_{EMRA} cells over the total CD4+ count was also higher in patients with current (4.86‰, IQR=0.25-11.43‰) and/or past (1.37‰, IQR=0.05-7.49‰) history of mycophenolate mofetil treatment than in patients with SLE having been receiving other treatments (0.05‰, IQR=0-0.68‰, p=0.005 and 0‰, IQR=0-0.63‰, p=0.013, respectively). Patients having been treated at least once with mycophenolate mofetil showed higher histone-specific naïve T cells

(2.94‰ CD4+, IQR=1.66-14.39‰) than patients with SLE treated with other therapeutic regimens (0.50‰ CD4+, IQR=0-1.34, p=0.048).

T cell activation assays

PBMC from 20 of the 53 subjects included in previous direct *ex vivo* immunophenotype studies (15 patients with SLE, including five with active disease and five HC; see Methods) were stimulated for 24h with peptide #1-5 as listed in **Table 6** according to their respective HLA-DRB1 profile. Unstimulated PBMC and PBMC stimulated with phytohaemagglutinin (PHA) served as controls. T cell activation was assessed by measuring the expression of CD69, OX40, CD40L, CD137 and HLA-DR on CD4+ and CD8+ T cells by flow cytometry. Double-positive CD69+OX40+ CD4+ subpopulations were used to validate classification of subjects into responders and non-responders to each stimulus. This approach was combined with measurement of 12 Th-related cytokines by flow cytometry using a cytokine bead array. Unstimulated PBMC from patients with active SLE showed significant signs of spontaneous cell activation and had higher IL6 levels (50.5 pg/ml, IQR=31.8-111.8) compared to patients with SLE in remission (6.8 pg/ml, IQR=3.4-13.5; p=0.002) and HC (13.5 pg/ml, 3.4-50.5; p=0.118). No significant differences were found in terms of CD8+ responses.

There were 7/13 activation marker responders to histone peptides among patients with SLE and 0/5 among controls (χ^2=5.115; p=0.044), while cytokine responses were detected in 6/13 patients with SLE and 1/5 HC. Consistently, patients with SLE had a median 1.01 (0.99-1.53)-fold increase in CD69 expression on CD4+ T cells after stimulation with histone peptides, while HC showed a relative decrease in CD69 expression (0.76-fold change, IQR=0.70-0.85 compared to no stimulation; p=0.022). Activation responses (either by surface marker expression and/or cytokine release) to histones tended to correlate with the detection of circulating histone-specific T cells by MHC-tetramer staining (χ^2=5.445; p=0.054). Furthermore, histone-responders by activation marker assays were more frequent among patients with beta-lactam allergy (5/6) than in patients without beta-lactam allergy (2/7; p=0.103). Activation marker expression following EBV-related peptides was observed in 3/9 patients with SLE and 1/5 HC. All these four subjects had at least one HLA-DRB1*11:01 allele (p=0.221). Cytokine responses were observed in 5/9 patients and 2/5 HC. All these subjects had

either a clinical history of EBV infection and/or evidence of EBV-specific T cells at immunophenotype analyses. Activation marker responses after penicilloylated albumin peptide stimulation were 6/8 among patients (with 7/8 having a history of beta-lactam allergy) and 2/5 among HC (**Table 13**).

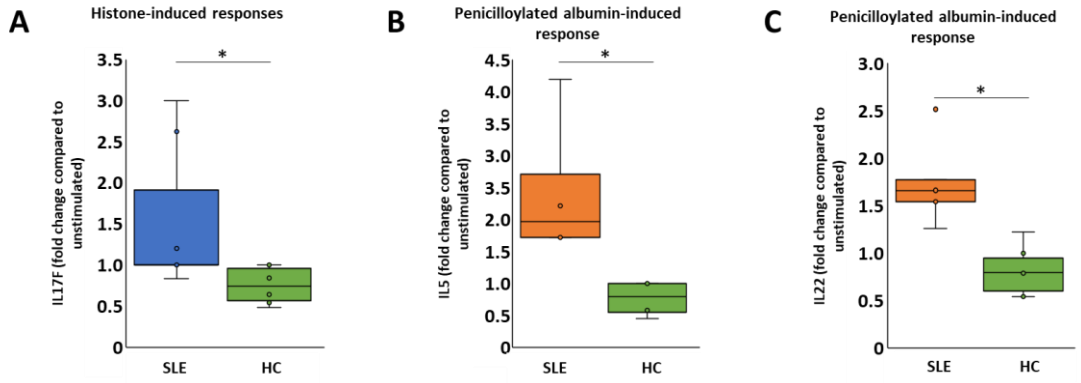
Patients with SLE and HC had diverging quantitative cytokine response profiles to peptide stimuli. Histone-induced IL17F levels were higher in patients with SLE than in HC. There was also a trend toward higher IL5, IL9 and IFNγ responses after histone peptide stimulation in patients than in controls (**Figure 12**). There were no significant differences in EBV-induced responses when HC were compared to patients with SLE. However, patients with active SLE had lower IFNγ, TNFα and IL22 EBV peptide-induced responses (p=0.044 for all three variables), when compared to HC. Subjects with HLA-DRB1*03:01 showed enhanced IL4 responses to EBV peptides compared to subjects with at least one HLA-DRB1*11:01 allele (p=0.033). Patients with SLE had enhanced IL5 and IL22 responses to penicilloylated albumin peptides compared to HC (**Figure 12**) and showed a trend towards higher penicillin-induced increases in IL17F secretion (p=0.088).

Table 13: reactogenicity of study epitopes

			Histone-specific responses				Beta-lactam-specific responses				EBV-specific responses			
Subject ID	HLA-DRB1	Diagnosis	Positive ADNA	Histone-specific T cells	Activation induced molecules	Cytokine response	Allergy to beta-lactams	Penicilloylated albumin-specific T cells	Activation induced molecules	Cytokine response	History of EBV infection	EBV-specific T cells	Activation induced molecules	Cytokine response
#1	03:01	SLE	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes
#2	11:01	SLE	Yes	Yes	No	No	No	No	NT	NT	Yes	Yes	No	No
#3	11:01	SLE	No	Yes	No	Yes	No	No	NT	NT	Yes	Yes	Yes	Yes
#4	11:01	SLE	Yes	Yes	Yes	No	No	No	NT	NT	Yes	No	Yes	No
#5	03:01	SLE	Yes	Yes	NT	NT	No	No	No	Yes	Yes	Yes	No	Yes
#9	11:01	SLE	Yes	Yes	NT	NT	Yes	Yes	No	Yes	ND	Yes	No	Yes
#11	11:01	SLE	Yes	Yes	Yes	Yes	No	No	NT	NT	ND	Yes	Yes	No
#12	03:01	SLE	No	Yes	Yes	No	Yes	No	Yes	NT	ND	No	No	No
#13	11:01	SLE	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	ND	Yes	Yes	Yes
#17	03:01	SLE	Yes	Yes	No	No	No	No	NT	NT	ND	No	NT	NT
#22	03:01	SLE	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	NT	NT
#25	03:01	SLE	Yes	Yes	No	Yes	No	No	NT	NT	ND	No	NT	NT
#28	03:01	SLE	Yes	No	No	No	No	No	NT	NT	ND	No	NT	NT
#30	03:01	SLE	No	No	Yes	Yes	Yes	Yes	Yes	Yes	ND	Yes	NT	NT
#32	03:01	SLE	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	ND	No	NT	NT
#47	11:01	HC	No	No	No	Yes	No	No	No	Yes	ND	Yes	No	Yes
#48	03:01	HC	No	No	No	No	No	No	No	No	Yes	No	No	Yes
#49	11:01	HC	No	No	No	No	No	No	Yes	No	ND	No	No	No
#50	03:01 and 11:01	HC	No	No	No	No	No	No	Yes	No	ND	Yes	Yes	No
#53	03:01	HC	No	No	No	No	No	No	No	No	ND	No	No	Yes

Comparison of reactogenicity data obtained with different methods for each antigen-of-interest and clinical phenotype. Activation-induced molecule responses were defined as the presence of higher levels of CD4+ CD69+ OX40+ after stimulation than in each respective negative control. Cytokine responses were defined as evidence of increased levels of at least one inflammatory cytokine after stimulation in comparison to each respective negative control. Abbreviations: HC: healthy control; ND: no data; NT: not tested; SLE: systemic lupus erythematosus.

Figure 12: cytokine responses in patients and controls



Boxplots showing variations in cytokine secretion compared to unstimulated conditions after exposure of PBMC from patients with SLE (blue and orange) and HC (green) to histone-derived peptides (panel A) and penicilloylated albumin peptides (panel B and C). Symbols: *: $p<0.05$.

DISCUSSION

Summary and significance of the acquired evidence

This study employed a translational design involving a combination of clinical, genetic and cellular biology data to characterise the multifaceted aspects of immune dysfunction in patients with SLE. The main findings obtained from clinical and experimental data are:

a) patients with SLE are at increased risk of allergic events compared to the general population;
b) allergic events co-occur with inflammatory flares and infections, suggesting that maladaptive responses to pathogens trigger or enhance tolerance loss towards self or environmental antigens;
c) dis-immune events including infections tend to cluster with specific HLA subtypes, which might be more permissive to aberrantly present innocent stimuli under inflammatory conditions and promote loss of tolerance towards them;
d) CD4+ T_{SCM} cells are expanded in patients with SLE, especially in the context of active disease;
e) antigen-specific CD4+ T cells recognising histone peptides and the penicillin major antigenic determinant correlate with a clinical history of positive ADNA and beta-lactam allergy, respectively;
f) patients with SLE bear increased levels of histone-, penicilloylated albumin-, EBV-specific CD4+ T cells compared to heathy and inflammatory disease controls of comparable demographics;
g) in patients with SLE, quantitative variations in these three T cell subtypes are correlated, mirroring what is observed clinically;
h) antigen-specific CD4+ T cell counts, especially in the T_{EM} compartment and in Th2 and Treg subsets tend to decrease during active SLE, likely reflecting their migration into target tissues;
i) consistent with immunophenotype data in unstimulated cells, immunogenic peptides from histones, EBNA and albumin bound to a penicilloyl group elicit T cell activation and cytokine release;
j) patients with SLE show more consistent responses to histone peptides than HC, although only a fraction of the expected ADNA/anti-histone positive cell

population mount significant cytokine and T cell activation responses, in line with previous studies (Lu et al., 1999); HC showed a decrease in cell activation following epitope exposure, possibly as the expression of tolerogenic responses;

k) penicilloylated-albumin triggers significant T cell responses in allergic patients with SLE, although also a non-negligible fraction of non-allergic HC shows detectable in vitro T cell reactivity, consistent with the hypothesis of natural penicillin reactogenicity in the general population (Azoury et al., 2018);

l) in response to histone and penicilloylated-albumin peptide, patients with SLE show a peculiar secretory profile, characterised by a mixture of pro-inflammatory and pro- allergic cytokines including IL5 and IL17; patients with active disease also have impaired cytokine release after EBV-derived peptide stimulation.

Interpretation of clinical findings

Clinical evidence from a well-characterised cohort of patients with long-term follow up reveals that autoimmune, allergic and infectious events tend to co-occur. This study also shows that the onset of an allergic diathesis tends to cluster with the onset of SLE and to be temporally related to an infectious event, consistent with previous reports (Cooper, Dooley et al., 2002, Strom, Reidenberg et al., 1994). The prevalence of allergy was higher in patients with SLE than in the reference population (Quercia et al., 2012), in agreement with previous reports (Hsiao et al., 2014, Sequeira et al., 1993). Furthermore, patients with early-onset SLE, who are generally characterised by a more severe rheumatologic profile (Ambrose et al., 2016), had increased chances of becoming allergic during their lifetime, suggesting that this subset of patients might present a deeper dysfunction of immune regulation, leading to accelerated sensitisation to self and environmental antigens. Allergy onset after SLE was associated with accelerated development of additional hypersensitivity reactions, which further supports a view of SLE deflecting allergy disease trajectories by promoting loss of tolerance to multiple antigens and/or, conversely, of allergen polysensitisation as a risk factor for SLE (Hsiao et al., 2014). Treatment with cyclosporine A or rituximab was associated with a higher allergy prevalence, consistent with the use of these drugs as second/third-line treatments in more

severe subjects (Fanouriakis et al., 2019, Gordon et al., 2018). Finally, accumulating allergic sensitisation events correlated with SLE damage accrual, suggesting that mechanism driving autoimmunity and hypersensitivity to environmental stimuli are deeply interwoven and/or part of a shared defect of immune regulation in patients with SLE.

Pharmacological agents were the most frequent culprit triggers of allergic events in patients with SLE, consistent with the notion of drug allergy as a peculiar feature of SLE phenotype (Parks et al., 2010, Petri & Allbritton, 1992, Sequeira et al., 1993, Wozniacka et al., 2003). Among drugs, antibiotics were most frequently represented, supporting a model in which infectious events leading to antibiotic prescription associate with drug sensitisation and, eventually, allergy, besides facilitating disease flares. Sulphonamide sensitisation has been reported as the major cause of drug allergy in patients with SLE (Petri & Allbritton, 1992). This study showed a significantly higher prevalence of beta-lactam allergy among patients with drug and antibiotic allergy. These data can easily be reconciled, since prevalence of individual culprit drug sensitisations is strongly correlated with prescription attitudes in a given clinical setting, with sulphonamides being progressively less employed in recent years (Strom, Schinnar et al., 2003). Consistent with this principle, NSAIDs and immunosuppressants were relatively more frequent as culprit drugs at prospective (post-SLE onset) than retrospective analyses.

Genetic evidence

Genetic characterisation of the HLA-DRB1 repertoire confirmed the strong association between HLA-DRB1*03:01 and SLE and showed a high prevalence of HLA-DRB1*15:01 in patients with SLE, consistent with the literature (Chung et al., 2014, Deng & Tsao, 2010, Diaz-Gallo et al., 2021, Morris et al., 2012, Teruel & Alarcon-Riquelme, 2016a). HLA-DRB1*11:01 was also highly represented among patients with SLE. Although previous data failed to show a clear association with SLE diagnosis, this finding is still consistent with evidence of association with other connective tissue diseases (Mammen, Gaudet et al., 2012, O'Hanlon, Carrick et al., 2006) and with the observations of (Miyagawa, Shinohara et al., 1997) in which an increased prevalence of HLA-DRB1*07:01, 15:02 was also detected among patients with TAK. Despite this latter condition being usually regarded as a class I MHC-related disease (Carmona et al., 2017),

associations with these two alleles and TAK have been reported by other authors (Dong et al., 1992, Lv et al., 2015). HLA genotypes not only constitute a potential risk factor for SLE, but also might correlate with discrete phenotypes, including the likelihood of experiencing hypersensitivity reactions and infectious events. To this latter regard, my results support a potential protective role of HLA-DRB1*07:01 towards multiple infectious threats (Amanzadeh, Amirzargar et al., 2012, Novelli, Andreani et al., 2020, Starshinova, Dovgalyuk et al., 2018, Wu, Wang et al., 2004) and suggest a potential role of HLA-DRB1*15:01 in modulating COVID-19 risk (Novelli et al., 2020), besides its known association with SLE and with enhanced herpesvirus responses (Kachuri, Francis et al., 2020, Teruel & Alarcon-Riquelme, 2016b).

Correlation of genotype information with clinical data also showed that HLA-DRB1*11:01 is associated with enhanced risk of allergy in patients with SLE, consistent with existing evidence in the literature from non-SLE populations (Bharadwaj, Illing et al., 2012, Park, Ahn et al., 2012, Quiralte, Sanchez-Garcia et al., 1999, Zhao, Zhao et al., 2019a). In this study cohort, HLA-DRB1*11:01 was associated with higher infection rates, further supporting a potential role for this specific HLA subtypes in shaping multiple aspects of immune dysfunction in patients with SLE. In line with this view, HLA-DRB1*11:01 was also associated with distinct cytokine responses after PBMC exposure to EBV-derived peptides.

General and antigen-specific T cell responses

Memory and functional characterisation of the T cell repertoire showed expansion of total T_{SCM} and T_{EM} cells in patients with SLE, consistent with data from SLE and other inflammatory disorders (Cianciotti et al., 2020, Lee et al., 2018, Piantoni et al., 2018). Abnormalities in primary differentiation and secondary activation of memory cells might account for relapse of autoimmune manifestations, spread of sensitisation towards cross-reactive antigens as well as persistent failure in infection control (Agmon-Levin et al., 2012, Fritsch et al., 2006). Total Th subpopulations number did not differ significantly among patients and controls (despite a trend towards higher Th2 counts in patients with SLE), in line with the literature and consistent with the limited role of relatively non-specific markers in adapting to SLE clinical/pathophysiological heterogeneity (Kubo et al., 2017).

Ex vivo visualisation of histone-, penicilloylated albumin- and EBV-specific CD4+ T cells through MHC multimers showed that all three biomarkers had a good diagnostic consistency with the clinical phenotype. Low levels of positive autoreactive and allergen-reactive cells were detected in a minority of non-SLE subjects and non-allergic subjects, respectively. Relatively higher rates of potential false-positive results were detected with T cell activation assays, in line with previous observations with penicilloylated albumin peptides and autoantigens (Abdirama et al., 2021, Azoury et al., 2018, Nhim et al., 2013). These data further support the hypothesis of (low-frequency) natural autoreactive and allergen-sensitive T cells being part of the physiological T cell repertoire of healthy individuals and point to the need for combined information on their functional behaviour to characterise their pathogenicity.

Histone-specific T cell responses

Histone-specific T cells were significantly more abundant in patients with SLE and correlated with a positive ADNA profile. Furthermore, histone-specific CD4+ T cell levels were affected by disease activity with more significant decreases in the T_{EM}, Th2 and Treg compartments, suggesting defective anti-inflammatory responses leading to facilitated peripheralization of effector cells into target tissues (Abdirama et al., 2021, Dolff et al., 2010, Yang et al., 2009). Notably, no significant correlation was found among histone-specific T cells and ADNA titres at time of sampling. Although the interpretation of these results is partially biased by the inclusion of patients' laboratory data from heterogeneous sources, reflecting the complexity of "real-life" clinical practice, these findings might suggest that fluctuations of ADNA titres might be uncoupled from variations in histone-specific T cell counts. Therefore, these two markers might have distinct, possibly complementary roles in monitoring disease activity, also in light of the insufficient ability of ADNA to thoroughly track active SLE manifestations (Conti et al., 2015, Isenberg, Manson et al., 2007). Further studies with homogeneous ADNA quantitation are, however, required to definitely confirm this hypothesis.

There was no clear correlation between histone-specific T cell counts and prednisone dose. Immunosuppression also had relatively little impact on histone-specific T cell counts and, most significantly, did not associate with depletion of these cells, possibly further strengthening the diagnostic utility of antigen-specific T cells as biomarkers and

supporting their pathogenic role in disease maintenance. Mycophenolate mofetil was the most frequent immunosuppressant in the study SLE cohort and its use associated with significantly higher levels of histone-specific CD4+ T cells, possibly with a more enhanced effect on naïve, T_{EM} and T_{EMRA} cells. These data suggest that mycophenolate mofetil might possibly exert more prominent anti-inflammatory rather than cytotoxic effects in patients with SLE, preventing flare-specific migration of pathogenic antigen-specific T cells into target tissues without dampening their development and proliferation (Allison & Eugui, 2005). Along with this line, molecularly-targeted treatments based on patient antigen-specific T cell profiles might complement current therapeutic strategies.

Consistent with clinical evidence of reciprocal relations between active SLE, infectious stimuli and allergic events, exploratory analysis on the reciprocal relations among trends of antigen-specific T cells showed that histone-specific T cell counts were correlated with EBV-specific T cell counts. EBV-specific cells in turn, could also be correlated with penicilloylated albumin-reactive cells, especially under active disease conditions. Consistently, T cell activation assays with histone peptides and penicillin allergy major antigenic determinant prompted complex cytokine responses encompassing a mixture of canonical pro-inflammatory and pro-allergic mediators including IL17 and IL5.

EBV-specific T cell responses

Similar to histone-specific T cells, EBV-specific T cells were also quantitatively expanded in patients with SLE compared to TAK and HC (despite comparable frequencies of EBV-positive subjects among the three groups) and decreased during active disease with a possible prominent role of T_{EM} and Treg cells. Consistent with the reciprocal correlation among the three types of immune dysfunction, patients with allergy to beta-lactams also showed reduced EBV-specific T_{EM}. In contrast to histone-specific CD4+ T cells, a significant activity-dependent decrease in EBV-specific CD4+ T_{SCM} cells (which were generally more represented in patients with SLE than in controls) was observed in concomitance with alterations of the Th17 subpopulations. Reduced EBV-specific CD4+ T_{SCM} cell counts might indicate progression of cell differentiation into effector cells in the setting of EBV reactivation with concomitant inflammatory activity (James, Kaufman et al., 1997). More intriguingly, EBV-specific CD4+ T_{SCM} drop in

concomitance with active disease might suggest the occurrence of mis-differentiation events favoured by molecular mimicry among EBV and self components (such as the Smith antigen) contributing to antigen spreading and pathological autoreactivity (James et al., 1997, Monneaux & Muller, 2002). Further evidence, including cross-stimulation of sorted antigen-specific T cells with incongruous stimuli, is however needed to validate this hypothesis. Expansion of EBV-specific CD4+ T cells in SLE has been reported by some authors (Kang et al., 2004), but unconfirmed by others (Draborg et al., 2014) using indirect detection techniques. Indeed, T cell activation assays in this study showed defective cytokine responses in patients with active disease, in line with previous reports (Berner, Tary-Lehmann et al., 2005, Draborg et al., 2014, Draborg et al., 2016).

Penicilloylated albumin-specific T cell responses

Penicilloylated albumin-specific Th2 cells were particularly expanded in patients with SLE and beta-lactam allergy, consistent with the higher prevalence of canonical, IgE-mediated, immediate-type rather than delayed-type hypersensitivity reactions linked to beta-lactam allergy in the general population (Mirakian et al., 2015). Treg and T_{EMRA} subpopulations were also prominently expanded among patients with beta-lactam allergy, possibly suggesting that while allergic sensitisation might be favoured by excessively permissive presentation of innocuous antigens during infectious/inflammatory flares, persisting allergen-induced inflammation is relatively more efficiently regulated after withdrawal of the inciting stimulus. Nonetheless, expanded naïve CD4+ T cells within the penicilloylated albumin-specific population suggest that these patients might be part of a subgroup of predisposed individual with intrinsic tendency to become sensitised to this type of drugs, as previously suggested (Azoury et al., 2018, Nhim et al., 2013).

General considerations

Taken together, these data might fit with a pathogenic model where disease flares are boosted or triggered by aberrant antimicrobial responses possibly delaying pathogen clearance and promoting sensitisation to bystander exogenous moieties such as drugs. The exact pathophysiological mechanisms accounting for misdirected T cell function towards self and environmental stimuli are, however, still unclear and will require additional research. A recent study addressed potential causes of T cell dysfunction in a

mouse model characterised by the development of autoantibodies against histones and allergy-like production of class E immunoglobulins. The authors found that lack of neuritin secretion by T follicular regulatory cells was associated with multidirectional immune dysregulation (Gonzalez-Figueroa, Roco et al., 2021). This molecule constitutes an attractive candidate to account for coexistent deregulation of T cell responses towards multiple antigens in patients with SLE. Anergy-related effective maintenance of regulatory T cell populations towards self antigens, preventing T_{EM} differentiation and eventual migration into target tissues can also have a role in this setting (Kalekar, Schmiel et al., 2016, Morikawa, Ohkura et al., 2014). Consistently, data from the present study show that autoantigen-specific T cells can be detected at very low levels even in HC, but are numerically expanded and functionally uncontrolled in patients with SLE, where histone-specific Treg functions are hyperbolically correlated with SLEDAI-2K.

Strengths and limitations

This study constitutes the first attempt to apply a translational approach based on robust clinical grounds and integrated with genetic and T cell function data to test the existence of evidence supporting a generalised dysfunction of the immune response towards pathogens, autoantigens and environmental antigens in SLE. Clinical evidence was acquired both retrospectively and prospectively on a relatively large cohort of well-characterised patients with SLE, which had the advantage of both cross-validating clues acquired from patients' history with subsequent clinical events and obtaining a comprehensive view of the interaction of infections, allergic events and autoimmune manifestations over time.

Clinical data

Epidemiological data from patients with SLE were compared with evidence in the general population from the literature and public databases, which might constitute a limitation given the methodological discrepancies among different studies. Data from public sources could not be used as reliable comparators to test differences in individual trajectories of allergic sensitisation accrual over time. General population data might also not entirely be appropriate to compare with SLE demographics and do not take into account the confounding effect of SLE treatments towards the risk of allergic sensitisation

and infection susceptibility. Identifying proper controls for allergic and infectious manifestations is, however, not straightforward, as these events are 1) point-like rather than chronic (in addition, allergic reactions are also relatively infrequent in a subject's individual history); 2) non-homogeneous; 3) unpredictable by means of clinical or genetic factors in the general and/or healthy population to the best of current knowledge; 4) not part of mass screening or follow up programmes. Therefore, very large, long-term population-based studies targeted on allergic and infectious events would be required to thoroughly validate the epidemiological results of the present study. Patients with SLE might also show disproportionately high drug allergy rates due to the frequent need to use drugs to combat inflammatory or, more frequently, infectious complications. Along with this line, healthy subjects *stricto sensu* might not have constituted a proper comparator to dissect the peculiar features of allergy in SLE, as they should virtually never had been exposed to inciting stimuli (that is drugs). On the other hand, having considered relatively healthy subjects with scanty clinical history as a potential comparator group would have disclosed additional dilemmas on the extent of pathological issues that can be tolerated for purposes of classification (Marchesini, Marchignoli et al., 2017). Finally, restricting the analysis to a single control disease or a limited set of potential control diseases might have introduced additional biases in terms of comparability of too different treatment regimens or, in the opposite way, too similar therapeutic strategies reflecting too similar pathogenic backgrounds. Taking all these considerations together, the use of public data from the general population, including healthy and unhealthy subjects might be intended as a potential compromise approach to explore potentially unique features of allergy in SLE in a feasible and minimally biased way. Indeed, non-selected control groups have been employed to assess allergy features both in cohort and population studies focused on SLE and rheumatic disorders (Hsiao et al., 2014, Kronzer, Crowson et al., 2019).

Delayed diagnosis is unfortunately still an incompletely resolved issue in the management of patients with SLE and might have further promoted enhanced exposure to a multitude of inappropriate drugs in the early phase of the disease (Oglesby, Korves et al., 2014). Conversely, as anticipated above, a lower prevalence of drug allergy might be expected in healthy subjects and patients with other disorders due to the overall reduced frequency of drug use. This in turn can be attributed to the fact that infectious and allergic events are expected to occur independently and without additional

relationships with autoimmune/inflammatory events in these individuals. By contrast, the co-occurrence of autoimmune, infectious and allergic events in this study was higher than expected from a random combination of independent events, suggesting that immune dysfunction mechanisms due to active disease directly affect allergy risk rather than simply augmenting the number of observed events by expanding the number of potential observations (that is situations in which patients had to use drugs).

Experimental data

T cell analyses were designed to address HLA-DRB1-restricted responses to selected peptide epitopes from autoantigens, allergens and antigens from infectious agents. In this context, patients with SLE were compared with healthy subjects and patients with TAK of comparable age and sex. The choice of TAK as a comparator group is not common in the literature because of the rarity of this disease. Nonetheless, TAK constitutes an ideal model for a T cell-dependent disease (Brack et al., 1997, Weyand et al., 1994) preferentially affecting young women and causing systemic inflammation as in SLE, while lacking SLE/connective tissue disease-specific mechanisms of immune dysfunction such as IFNα dysregulation and anti-nuclear immunity and being therefore unlikely to cause overlap syndromes with SLE. Epitope-bound MHC tetramers were used for selectively detect autoantigen-, allergen- and pathogen-specific CD4 T cells in the peripheral blood of patients with SLE and controls and characterise their function. Using class II MHC tetramers to track CD4+ T cells in autoimmune conditions has been attempted by a minority of authors due to technical and cost challenges (Cianciotti et al., 2020, James et al., 2014). In fact, in contrast to class I MHC, class II MHC is highly polymorphic in the general population, prompting to the need for high-resolution HLA genotyping of a large number of subjects as in the case of this study. Furthermore, in contrast to CD8+ responses (especially towards viral targets), autoreactive CD4+ responses are generally reduced in size and characterised by lower-affinity TCR expression, leading to low intensity MHC tetramer signalling and the need for countermeasures such as protein kinase inhibitors to prevent TCR internalisation (Dolton, Tungatt et al., 2015, Massilamany, Upadhyaya et al., 2011). By using MHC tetramers for *ex vivo* visualization of antigen-specific CD4+ cells, this study provides the first direct evidence of a) autoreactive T cells selectively recognising chromatin-related antigens in

SLE; b) antigen-specific T cells able to recognise penicillin allergy major antigenic determinant in subjects with beta-lactam allergy, possibly overcoming the drawbacks of classical *in vitro* studies, where cultured and stimulated T cells might not reflect the actual *in vivo* phenotype (James et al., 2014).

These data were strengthened by the frequent detection of EBV-reactive cells in patients and controls, confirming previous evidence and consistent with the high rates of EBV infection in the general population. Adding on existing literature on T cell responses against autoantigens, the use of MHC-multimer direct T cell tracking disclosed unprecedented clues on the prevailing differentiation stage, polarisation and dynamics over time of antigen-specific T cells against multiple targets in the circulating blood of patients with SLE. Furthermore, this study provides the first evidence of potential reciprocal correlations among T cells recognising autoantigens, allergens and pathogens, possibly providing a pathophysiological correlate for clinical observations. Finally, genetic analysis on a relatively large sample of patients and controls corroborated clinical observations and previous evidence in the literature, besides showing novel potential associations with specific clinical aspects of SLE. However, interpreting these data requires careful considerations of multiple limitations, which in turn are mainly explainable with the exploratory nature of this research. The design of this study excluded non-peptide and non-HLA-DRB1 restricted epitopes from analysis. This is particularly relevant for DNA-targeted immunity which was assumed to be surrogated by anti-histone immunity (Schett, Smole et al., 2002). In contrast to indirect antigen-specific assays employing peptide libraries (Abdirama et al., 2021, Tesch et al., 2020, Zhao et al., 2019b), this study focused on a relatively limited set of peptides. Less frequent but potentially specific antigens such as the Smith antigen were excluded (Zhao et al., 2019b), preventing a comprehensive view of the broad autoantigen repertoire characterising SLE. In a similar way, the choice of penicilloylated albumin is insufficient to address the whole spectrum of beta-lactam allergy, as many subjects can become sensitised to side chains of beta-lactam drugs, rather than to the penicilloyl group. Besides, allergic responses to non-drug-related antigens and other less frequent allergens could not be addressed by this study. Choosing EBNA1 and 2 as the reference pathogen-related antigens also implies some limitations. In fact, despite having consistently been implicated in SLE flares, EBV causes a chronic infection with potential reactivations instead of being responsible for

acute events. Therefore, data regarding EBV-specific T cell dynamics can likely only partially surrogate the effects of pathogen-related perturbations to the immune response in patients with SLE, pointing to the need for further evidence on other microbial agents such as influenzaviruses. Furthermore, opting for a limited choice of EBV-related peptides prevented a comprehensive analysis of individual variations in the profile of anti-EBV responses and subsequent pathways of SLE flare induction. The absence of complete EBV serological data from the study subjects constitutes an additional limitation to the interpretation of EBV-specific T cell responses. Finally, although attention was paid to select blood samples from patients with as low immunosuppression as possible, treatment profiles were not homogeneous among patients, adding potential confounders to the evaluation of antigen-specific T cell dynamics. Nonetheless, as differences among patients taking and not taking immunosuppressant were minimal, data from this study might suggest that antigen-specific T cell tracking in SLE can robustly be translated into the complexity of routine clinical practice.

T cell activation assays after incubation with peptides employed for MHC-peptide multimer studies provided further evidence supporting the biological relevance of T cell responses targeting these antigens in the pathogenesis of SLE. In contrast to direct antigen-specific T cell tracking analyses, low-grade responses were more frequent in this set of experiments, which prevents a detailed insight in the kinetics and qualitative features of T cell response to direct stimulation with relevant triggers. To this regard the choice of a single-point 24h-time incubation protocol might have been insufficient to comprehensively address surface marker and cytokine dynamics with peaks at different timepoints. Further research is also required to understand whether distinct peptide doses might elicit different T cell responses.

In addition to previous points, expansion of the patient sample size employed for *in vitro* studies will probably provide more informative data and better conform to SLE extreme clinical and pathophysiological variability. Larger studies are also required to address prospectively the potential significance of antigen-specific T cell dynamics over longer observation timeframes, also in light of patient heterogeneity in terms of quantitative and qualitative responses to specific antigens.

Future perspectives

Identifying circulating antigen-specific CD4+ T cells of potential clinical relevance for autoimmune, allergic and infectious manifestations opens multiple potential perspectives for future research and applications. In terms of diagnostics, antigen-specific T cells might become part of the tools employed to profile patients with SLE at diagnosis and monitoring the disease course over time. To this regard expansion of the HLA-multimer and peptide panel (for example to include the SLE-specific HLA-DRB1*15:01, which was unfortunately too infrequent in the subject sample included in this study to be used for *in vitro* studies) in the setting of future research would increase the feasibility of translating antigen-specific T cell tracking in the rheumatologic clinical practice (Abdirama et al., 2021, Tesch et al., 2020). These approaches might also synergise with patient stratification algorithms based on HLA- and antibody profiling and identify subsets of subjects with higher expected success rates following specific treatments (Diaz-Gallo et al., 2021).

The use of tolerogenic approaches employing repeated or regulated exposure of target antigens to affected subjects is widely used in routine allergy practice (Nakagome & Nagata, 2021), while less robust evidence has so far been acquired for autoimmune disorders. MHC multimers loaded with relevant peptide autoepitopes and embedded in iron oxide nanoparticles might widen the therapeutic armamentarium for multiple diseases such as type I diabetes and rheumatoid arthritis (Clemente-Casares et al., 2016). In the setting of SLE, treatment with autopeptide-bound MHC multimers might also synergise with enhanced immunomodulatory approaches based on lipid nanoparticle delivery of antimalarials (Diao, Tao et al., 2019, Serra & Santamaria, 2020, Stevens, Crist et al., 2020). In turn, the use of flow-cytometry assays based on the use of MHC multimers might identify patients with the ideal profile to receive immunomodulating antigen-specific treatments.

Chimeric antigen receptor T (CAR-T) cell therapy is a rapidly emerging potent tool for the treatment of otherwise intractable haematological malignancies. Engineered CAR-T cells exploits T cell selectivity and amplitude of functions to accurately and persistently suppress selected target cells or remodulate inflammatory responses to a given epitope. The efficacy of CAR-T cell therapies in animal models of autoimmunity constitutes an attractive perspective for patients with autoimmune diseases such as SLE (Beheshti,

Shamsasenjan et al., 2022, Jin, Han et al., 2021, Mougiakakos, Kronke et al., 2021). However, besides high costs, extensive interindividual variability among subjects in terms of autoantigen sensitisation profiles constitutes a major limitation for the application of this innovative approach in patients with autoimmune diseases such as SLE, in contrast with clonal disorders, where a fixed target antigen can be relatively more easily identified. Direct *ex vivo* visualization and characterisation of antigen-specific T cells has long been proposed as a tool to optimise vaccine and allergen immunotherapy design based on patient characteristics and expected T cell reactivity (Archila, DeLong et al., 2014, Munz, Bickham et al., 2000). Routine use of MHC-tetramer based approaches for an extended panel of key epitopes for the pathophysiology of SLE might enable patient profiling for effective and tailored CAR-T cell treatments.

Conclusions

Patients with SLE are susceptible to autoimmune, allergic and infectious events, which tend to co-occur, suggesting a shared pathophysiological mechanism accounting for multidirectional immune dysfunction. To this regard, this study provides the first direct evidence in SLE of HLA-restricted antigen-specific CD4+ T cells recognising epitopes from key autoantigens, allergens and chronic infectious agents and correlating with the clinical phenotype. Data from T cell differentiation and polarisation phenotyping together with functional tests indicate that these cells undergo dynamic variations mirroring fluctuations in disease activity, which might implicate their potential future use to complement existing diagnostic tools and guide and/or integrate current therapeutic weaponry.

MATERIALS AND METHODS

Patients and controls

This translational study is based on a combination of clinical and experimental data. The general design of the study is depicted in **Figure 13**. Upon informed consent under the Autoimmuno-mol research protocol (approved by the Institutional Review Board of IRCCS Ospedale San Raffaele, ref. 2/2013/INT) 190 patients with SLE and 178 control subjects including 94 healthy controls and 84 patients with TAK were enrolled. SLE was defined according to either the revised 1997 ACR criteria or the 2012 SLICC criteria (Hochberg, 1997, Petri et al., 2012). The diagnosis of TAK was established by the 1996 Sharma's criteria (Sharma, Jain et al., 1996). TAK was selected as an inflammatory control group, being a T cell-dependent disease with similar demographic features compared to SLE. Peripheral blood mononuclear cells (PBMC) were collected from these subjects during routine clinical procedures or visits along with clinical data encompassing disease history, disease manifestations, comorbidities, past and current treatments and the results of routine laboratory tests at time of analysis. Patient selection for *in vitro* studies was based on availability of sufficient PBMC samples, minimisation of discrepancies in terms of confounders (age, sex, corticosteroid and immunosuppressant doses, concomitant non-disease-related clinical events such as trauma or cancer etc.) among groups and on the results of HLA genotyping (see below). Patients with active disease underwent blood sample collection before having been started on new treatments. Fifty-three subjects (32 SLE, 11 TAK, 10 HC) were included in T cell analyses. Five patients with SLE in remission were retested with samples obtained during disease activity. One HC bearing both HLA-DRB1 alleles of interest was tested twice, employing both sets of MHC-tetramers and peptide sets.

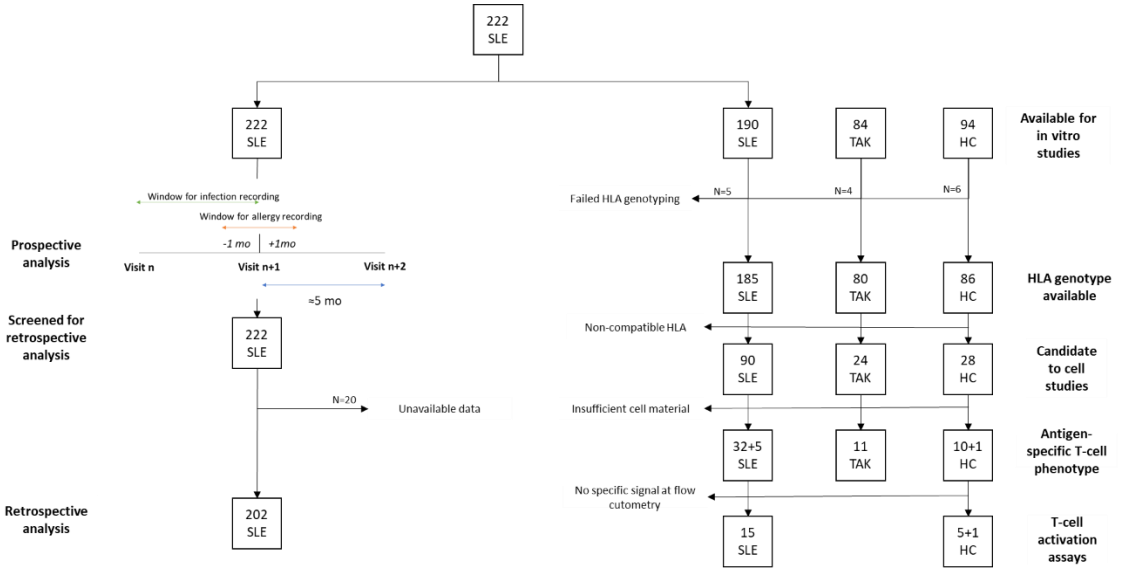
Upon informed consent, a total of 222 patients with SLE including those enrolled in the Autoimmuno-mol protocol were also enrolled in a prospective study focusing on clinical features of patients with multiple immune-mediated diseases (PanImmuno research protocol, approved by the Institutional Review Board of IRCCS Ospedale San Raffaele, ref. 22/INT/2018). Under the frame of this protocol, patients with SLE were cross-sectionally enrolled and prospectively followed up in concomitance with routine clinical evaluations occurring at 3-6-month intervals. Data regarding disease features at time of each visit, the results of laboratory tests employed for clinical assessment

(including complete blood cell count, erythrocyte sedimentation rate and C-reactive protein measurement, renal and liver function tests, complement C3 and C4 levels and ADNA titres), disease activity and disease-related damage, ongoing and new treatments, recent infections and/or allergic reactions were all collected through an in-house software (Clinimatrix®, Italian Society for Authors and Editors, SIAE, registration number 013059-D012254) which I developed based on Microsoft Excel® (Ramirez et al., 2019b, Ramirez, Rocca et al., 2021). Five categories of ADNA were defined (borderline, low, moderate, high, very high) based on the attending Physician's interpretation of the laboratory test results. For ADNA measured by indirect immunofluorescence in a dilution range from 1:20 to over 1:640, the following classes were identified: 1:20 – borderline, 1:40-80 – low, 1:160-320 – moderate, 1:640 high, >1:640 – very high.

Allergic reactions were defined as "hypersensitivity reactions initiated by immunological mechanisms" as per the World Allergy Organization/European Academy of Allergy and Clinical Immunology (WAO/EAACI) consensus definitions (Pawankar, Canonica et al., 2013). Allergic reactions occurring within one month before or after each study visit were considered significant for prospective analysis. Similarly, occurrence of at least one infection requiring systemic antimicrobial therapy or absence from work in the timeframe between each visit was set as the criterion to record infectious events during follow up visits since enrolment. Data regarding COVID-19 infection rates during 2020 were also collected under the same protocol and analysed separately.

In parallel with prospective analyses, data regarding demographics, the course of SLE from disease onset, comorbidities and allergic history were also collected. Regarding allergy history, the same definitions of allergy as per above were applied. For purposes of classification (both in prospective and retrospective studies), only clinical events having at least one of the following characteristics were considered as allergic reactions: a) direct observation of the reaction by a Physician; b) laboratory or clinical evidence of allergic sensitisation through specific IgE testing, basophil activation test, skin prick tests, patch tests or intradermal tests in correlation with a consistent clinical history; c) ability of patients to recall sufficient clinical details being consistent with an allergic reaction as evaluated by an Allergist.

Figure 13: general study flow chart



This picture summarises the general design of the present study, which involved 222 patients with SLE and 178 controls. The left side of the graphs reports the main characteristics of the clinical studies performed within the frame of this work. All patients were followed up prospectively over the course of >1200 outpatient visits occurring approximately every six months for each patient. Infectious events occurring within this timeframe were recorded along with allergic reactions taking place within one month before or after each outpatient visit. Extensive data on disease activity in terms of autoimmune manifestations and on treatments were also collected. Only 202 of the patients enrolled in the prospective study could recall and/or document with clinical charts details about allergic and infectious history before enrolment. On the right side of the chart, the progressive steps leading to the selection of patients suitable for *in vitro* studies is represented. A relatively large number of patients with SLE and controls was screened to define the feasibility of subsequent studies employing major histocompatibility (MHC) tetramers for antigen-specific T-cell detection at flow cytometry. Among these subjects, encompassing healthy controls (HC) and patients with Takayasu's arteritis (TAK), a subset with sufficient cell aliquots to perform flow cytometry and stimulation assays was identified. A total of 53 subjects (one bearing both HLA-DRB1 alleles-of-interest, tested twice) underwent antigen-specific T-cell phenotyping. Five patients with SLE were tested both during active disease and remission. Among the 53 subjects included in T cell studies, twenty (15 patients with SLE and five HC) were selected for T-cell activation assays based on the results of flow cytometry analyses.

Clinimetrics

In patients with SLE, disease activity was quantitated through a panel of validated measures including the SLE disease activity index 2000 (SLEDAI-2K), the British Isles Lupus Assessment Group (BILAG) 2004 score, and the European Consensus Lupus Activity Measurement (ECLAM) scale (Gladman et al., 2002, Isenberg et al., 2005, Mosca et al., 2000). Lupus low disease activity status (LLDAS) was used a surrogate

index of remission (Franklyn et al., 2016). Physician and patient impression on disease activity were quantitated through a 0.0-3.0 Physician Global Assessment score (PGA) and a 0-10 numerical rating scale respectively. Damage accrual was expressed as SDI (Gladman et al., 2000). Clinimetrics calculation and recording was performed through Clinimatrix®.

Disease activity in patients with Takayasu's arteritis was assessed through the Indian Takayasu disease Activity Score (ITAS-2010)(Misra, Danda et al., 2013). Patients were also categorised by Physician's impression into patients in remission, with smouldering disease, or with active disease.

Population data and bioinformatics

Epidemiological data including prevalence of allergic disorders and EBV infection in the general population were obtained from publicly available databases and by literature search through the National Center for Biotechnology Information and Google Scholar® tools. Specifically, the prevalence of self-reported history of allergy by age-groups in Italy was extracted from Eurostat (http://ec.europa.eu/eurostat/web/health/data/database, accessed in September 2019; data referred to 2014) and from the Italian National Institute of Statistics (ISTAT: www.istat.it, accessed in September 2019; data referred to 2017). Additional data were retrieved from a population study (Quercia et al., 2012) and integrated with further analysis on the same database (kind courtesy of Prof. Cristoforo Incorvaia). Data regarding general European demographics were also extracted from Eurostat. A comprehensive summary of these analyses is reported in **Appendix 1**. Expected frequencies of HLA genotypes in the general Italian population were retrieved from the Allele Frequency Net (Gonzalez-Galarza et al., 2019).

Blood and DNA samples

PBMCs were purified from whole blood samples collected into 6 ml EDTA containing tubes (BD Vacutainer®) through gradient separation with Lymphoprep™ and biobanked until use. Live PBMC or residual non-live white blood cells after PBMC extraction were used for DNA extraction. Sample processing and storage were performed by a dedicated staff at a centralised biobank facility (current Centro Risorse Biologiche, CRB) following the internal standard operating procedure IOS SIMT 101.

DNA extraction was performed with silica-membrane spin columns (Cat. #69506) or 96-well plates (Cat. # 69581) from Qiagen® according to the manufacturer's instructions, except for the use of distilled water for the final elution step and for storage. The amount of extracted DNA was checked by mass-spectrophotometry (Nanodrop 1000®, ThermoFisher, Massachusetts, USA).

HLA genotyping

A total of 380 subjects (190 patients with SLE, 190 controls) with available DNA samples were genotyped for their HLA-DRB1 profile. Specifically, high-resolution four-digits HLA typing was performed by sequence-specific oligonucleotide-primed polymerase chain reaction (PCR-SSO), using Histo-spot® DRB1 kits (Cat. No. 726040 and 726098), AstraFormedic, Milan, Italy. These experiments were performed by Dr Benedetta A. Mazzi and collaborators in the Immunogenetics Laboratory, HLA & Chimerism, Department of Immunohematology & Blood Transfusion of IRCCS Ospedale San Raffaele.

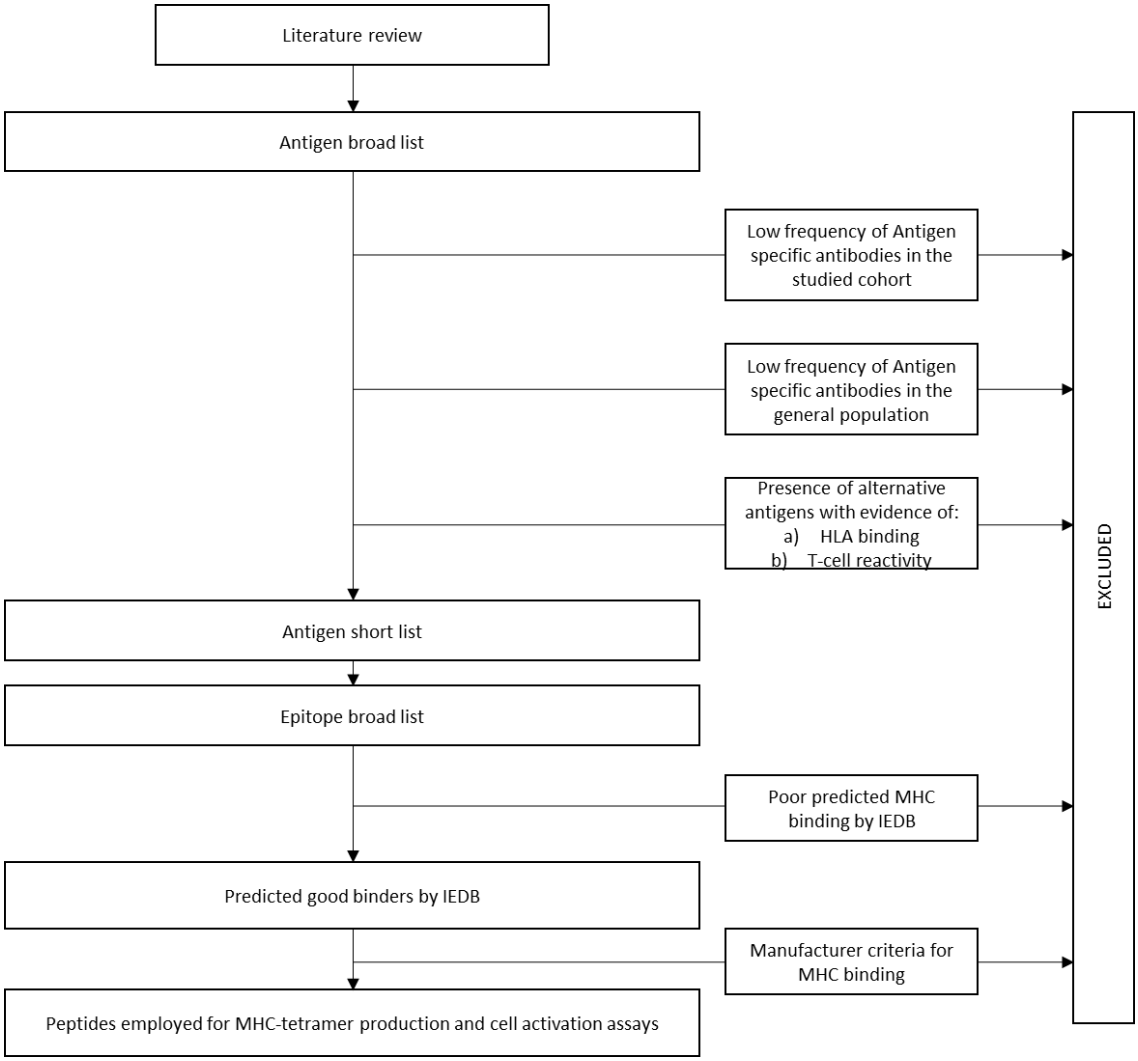
Peptides, antigens and epitopes of interest

In silico analyses

Based on the results of HLA typing (see above and **Appendix 2**) and on literature review, two HLA-DRB1 alleles (HLA-DRB1*03:01 and 11:01) were selected for further studies with the aim of obtaining data from a sufficient number of subjects with a pathogenically meaningful genetic profile. Specifically, expected HLA-DRB1 frequencies in the general population were retrieved from the Allele Frequency Net Database (Gonzalez-Galarza et al., 2019). This information was combined with previous studies reporting on potential associations of selected HLA genotypes with SLE (Arango et al., 2017, Bang et al., 2016, Ceccarelli, Perricone et al., 2015, Cruz, Shao et al., 2016, de Holanda, Klumb et al., 2018, Massa, Mazzoli et al., 2002, Morris, Fernando et al., 2014, Morris et al., 2012, Niu et al., 2015, Teruel & Alarcon-Riquelme, 2016b), infection susceptibility (Amanzadeh et al., 2012, Kawase, Tanaka et al., 2019, Muro, Mondejar-Lopez et al., 2013, Starshinova et al., 2018, Wu et al., 2004), and/or allergy (Azoury et al., 2018, Lara-Marquez, Yunis et al., 1999, Nhim et al., 2013, Padovan et al., 1997, Park et al., 2012, Quiralte et al., 1999).

Potential candidate peptides for MHC binding and cell stimulation were identified starting from a broad list of known autoantigens, allergens and antigens belonging to infectious agents (**Figure 14**). This selection was refined by ruling out candidate antigens whose corresponding antibodies had a low prevalence in the studied cohort or in the reference population. Further shortening of the initial list was performed by selecting antigens endowed with peptides having already shown evidence of a) efficient binding to the HLA-DRB1*03:01 and/or HLA-DRB1*11:01; b) ability to elicit T-cell responses in vitro in patients with SLE or other subjects against antigens with no such evidence in the literature when available. After this preliminary selection, full FASTA sequences of each antigen in the shortlist were retrieved from the U.S. National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/protein). These sequences were then inputted in the Immune Epitope Database (IEDB) and Analysis Resource (www.iedb.org), developed by the U.S. National Institute of Allergy and Infectious Diseases to identify potential T-cell epitopes restricted for the two MHC of interest. This tool integrates multiple algorithms for peptide-MHC binding prediction and provides a global percentile rank reflecting the likelihood of each sequence to bind a given MHC molecule (Paul, Lindestam Arlehamn et al., 2015). Only peptides showing a percentile rank below 25 were considered. More stringent criteria were applied after further counselling from the MHC tetramer manufacturer (Proimmune®) to include only peptides with a nonamer core fitting specific binding criteria.

Figure 14: flow-chart for selecting peptides for MHC-tetramers and cell activation assays.



This flow chart depicts the steps taken to narrow an initial list of potential candidate antigens and epitopes of possible significance for the study into a shortlist of peptides. Abbreviations: HLA: human leukocyte antigen; IEDB: Immune Epitope Database; MHC: major histocompatibility complex.

Peptide synthesis and reconstitution

The same peptide sequences employed for MHC-tetramer experiments were used for cell stimulation and were purchased as five-mg powders from Biomatik Corporation (Ontario, Canada). The penicilloylated PELLFFAKRYKAAFT human albumin peptide was produced as described by Scornet et al. (Scornet, Delarue-Cochin et al., 2016). One mg of powder was first tested for solubility in DMSO, then mixed to the remaining

amount and aliquoted. Specifically, 5 mg of each peptide were reconstituted in 250 µl DMSO and stocked at -80°C in aliquots of 10 µl until use. For cell stimulation, each 10 µl aliquot was diluted with 20 µl PBS to reach a final concentration of 6.7 µg peptide /µl PBS+DMSO. A volume of 1.5 µl of this solution was added to 500 µl of cell culture medium (see below), yielding an operative peptide concentration of 20 µg/ml. The total concentration of DMSO in the cell medium was therefore 0.1%, which is below the 0.5% threshold of cytotoxicity conventionally recommended for T cell experiments.

Tetramers

Peptide-bound MHC tetramers of HLA-DRB1*03:01 and HLA-DRB1*11:01 were purchased from ProImmune Ltd (Oxford, United Kingdom). The penicilloylated PELLFFAKRYKAAFT sequence was produced by Biomatik Corporation as described above and incorporated by Proimmune into HLA-DRB1*03:01 and HLA-DRB1*11:01 MHC tetramers. Phycoerythrin (PE)- and allophycocyianin (APC)-bound MHC tetramers were generated for histone and penicilloylated albumin peptides respectively. EBV-derived peptides were provided by Proimmune as 35µg/88µl HLA-DRB1*03:01 and HLA-DRB1*11:01 MHC-biotin monomers. Tetramerisation of MHC monomers was performed as follows, by adapting the protocol provided by ProImmune for tetramerising PE-bound MHC monomers and that of Lu et al. for fluorescein isothiocyanate (FITC)-bound MHC monomers (Lu, Yoo et al., 2019). FITC-streptavidin to MHC-monomer molar ratio was set to 1:4. Therefore, assuming that MHC monomers had a molecular weight of 58-65 kDa, a starting MHC amount of 0.569 nmol was calculated, which implied the need for 0.14 FITC-streptavidin nmol. A 500µg/ml FITC-streptavidin aliquot (Biolegend) was centrifuged at 14,000 g at 4°C for three minutes and then kept on ice protected from light. Ten µg FITC-streptavidin (>0.14 nmol) was then added in five steps separated by 15-minute intervals of incubation to the MHC monomer solution. The resulting 108µl FITC-MHC-tetramer solution was then further diluted in PBS to 400 µl total volume. To test whether unbound FITC-streptavidin could prompt non-specific FITC-positive signal on flow cytometry, 1 x 10^6 PBMC from healthy donors were stained with FITC-streptavidin up to 10-fold the concentration achieved in the FITC-MHC-tetramer solution. Unstained samples and samples stained with an anti-CD3-FITC

antibody (Biolegend) served as control tests. FITC-streptavidin fluorescence intensity was undistinguishable from the negative (unstained) control (**Appendix 9**).

Flow cytometry

Gating strategies

The following fluorochrome-bound antibodies were used for cell staining and phenotyping: anti-CD3-Pacific Blue (Cat #300431), anti-CD4-PE-cyanin7 (Cat #300512), anti-CD8-peridinin-chlorophyll-protein(PerCP)-cyanin 5.5 (Cat #344710), anti-CD45RA APC-cyanin 7 (Cat #304128), anti-CD62L AlexaFluor 700 (Cat #304820), anti-CD95 PE-Dazzle (Cat #305634), anti-CD3 Brilliant Violet 510 (Cat #317332), anti-CD127 PerCP-cyanin 5.5 (Cat #351322), anti-CCR4 PE-Dazzle (Cat #359420), anti-CCR6 Brilliant Violet 421 (Cat #353408), anti-CXCR3 APC-cyanin7 (Cat #353722), anti-CD69 APC-cyanin 7 (Cat #310914), anti-CD40L AlexaFluor 700 (Cat #310846), anti-CD137 PE (Cat #309804), anti-HLA-DR APC (Cat #307610), anti-OX40 FITC (Cat #350006; all from Biolegend) and anti-CD25 allophycocyanin-Alexa Fluor 700 (Beckman Coulter, Cat #A86356). In addition, vital staining was performed with Zombie Aqua™ (Biolegend, Cat #423101). Antibody and tetramer staining concentrations were optimised through preliminary tests with increasing reagent amounts. A stain index (SI) with the following equation was used to rank the resulting mean fluorescence intensities (MFI) according to their difference from negative (unstained) controls: $SI = \frac{MFI_{pos} - MFI_{neg}}{SD_{neg} \times 2}$ (SD=standard deviation; pos: positive sample, neg: negative control). Flow cytometry data were acquired and compensation performed through a Beckman Coulter Navios flow cytometer. Consistency among experiments in terms of fluorescence readings was checked by the use of Spherotech Spero™ rainbow calibration particles. Flow cytometry data were analysed with FCS Express version 7 and eventually exported to Microsoft Excel 2019 for further analysis and merging with clinical data. Absolute tetramer-positive cell counts were calculated as the number of CD4+ tetramer+ events after subtraction of CD4- tetramer+ events. Positive histone-, EBV- and penicilloylated albumin-reactive CD4+ T cell statuses were defined by diagnostic threshods based on ADNA status, EBV serology or history and beta-lactam allergy history, respectively (see Statistical analyses). Three staining panels were set up.

Panel A: lymphocyte differentiation

In this panel, PBMC from patients and controls were stained with Zombie Aqua™, anti-CD3-Pacific Blue, anti-CD4-PE-cyanin 7, anti-CD8-PerCP-cyanin 5.5, anti-CD45RA APC-cyanin 7, anti-CD62L AlexaFluor 700 and anti-CD95 PE-Dazzle antibodies with or without MHC-PE, MHC-FITC and MHC-APC tetramers. Preliminary tests revealed the presence of a large population of cells with coexistent strong FITC-MHC/PE-MHC fluorescence without dead cell exclusion. Back-gating this cell population on the vital stain histogram confirmed that these cells were all non-living (Staats, Divekar et al., 2019). Therefore, an exclusion gate for "spurious" events was created to eliminate the potential confounding effect of these cells on subsequent gate setting and analysis (**Appendix 10**). T cell subsets were further classified based on the gating strategy reported in **Table 14** (Cieri et al., 2015, Gattinoni et al., 2017, Kubo et al., 2017).

Table 14: gating strategy for lymphocyte differentiation analysis

Lymphocyte subset	Immunophenotype
T-lymphocytes	CD3+
T helper	CD4+
Naïve T helper	CD4+ CD45RA+ CD62L+ CD95-
Stem cell memory (T_{SCM})	CD4+ CD45RA+ CD62L+ CD95+
Central memory (T_{CM})	CD4+ CD45RA- CD62L+
Effector memory (T_{EM})	CD4+ CD45RA- CD62L- CD95+
Terminally differentiated (T_{EMRA})	CD4+ CD45RA+ CD62L-
T cytotoxic	CD8+
Naïve T cytotoxic	CD8+ CD45RA+ CD62L+ CD95-
Stem cell memory (T_{SCM})	CD8+ CD45RA+ CD62L+ CD95+
Central memory (T_{CM})	CD8+ CD45RA- CD62L+
Effector memory (T_{EM})	CD8+ CD45RA- CD62L- CD95+
Terminally differentiated (T_{EMRA})	CD8+ CD45RA+ CD62L-

Panel B: polarisation

In this panel, PBMC from patients and controls were stained with anti-CD3 Brilliant Violet 510, anti-CD127 PerCP-cyanin 5.5, anti-CCR4 PE-Dazzle, anti-CCR6 Brilliant Violet 421, anti-CD25 APC-Alexa Fluor 700 and anti-CXCR3 APC-cyanin 7 antibodies, with or without MHC-PE, MHC-FITC and MHC-APC tetramers. In the absence of vital

stain in this panel, the exclusion gate defined for panel A was applied with the same parameters to panel B. Functional polarisation cell subsets were defined as reported in **Table 15**, based on the literature (Becattini, Latorre et al., 2015, Kubo et al., 2017, Zhong et al., 2018).

Table 15: gating strategy for T cell polarisation analysis

Lymphocyte subset	Immunophenotype
T-lymphocytes	CD3+
T helper	CD4+
Regulatory	CD4+ CD25bright CD127low
T helper 1	CD4+ CXCR3(=CD183)+ CCR6 (=CD196)- CCR4(=CD194)-
T helper 17	CD4+ CXCR3(=CD183)- CCR6 (=CD196)+ CCR4(=CD194)+
T helper1*	CD4+ CXCR3(=CD183)+ CCR6 (=CD196)+ CCR4(=CD194)+
T helper 2	CD4+ CXCR3(=CD183)- CCR6 (=CD196)- CCR4(=CD194)+

Panel C: T cell activation

This panel encompassed Zombie Aqua™ staining along with anti-CD3-Pacific Blue, anti-CD4-PE-cyanin7, anti-CD8- PerCP-cyanin 5.5, anti-CD69 APC-cyanin 7, anti-CD40L AlexaFluor 700, anti-CD137 PE, anti-HLA-DR APC and anti-OX40 FITC antibodies. A subpanel with staining for CD3, CD4 and CD8 was also set for positive (phytohaemagglutinin, PHA-exposed) controls to determine the background fluorescence for each activation marker. Double-positive CD69+OX40+ CD4+ subpopulations were used to classify subjects into responders and non-responders to each peptide/control stimulus (Pallikkuth, Williams et al., 2021, Xiaoyan, Pirskanen et al., 2006).

Staining protocol for unstimulated T-cell phenotyping

For flow cytometry assays cells were gently thawed and resuspended in 15 ml RPMI medium (ThermoFisher, Gibco, Cat#72400-021) with 1/1000 gentamicin and 10% FBS (culture medium). After centrifugation at room temperature, 1500 rpm for 10 minutes, the supernatant was disposed to avoid DMSO contamination and the cell pellet resuspended in 8 ml culture medium. After overnight incubation at 37°C, 5% CO_2, 1 x 10^6 cells per tube were placed in four polypropylene tubes per patient corresponding to panel A for differentiation studies, panel B for T polarisation phenotyping with and

without peptide-bound MHC tetramers. After washing with 2 ml washing solution (PBS + 2% bovine serum albumin) per tube, vital staining was performed with Zombie AquaTM (panel A). After 10-minute incubation at room temperature, dasatinib 10 μM 1μl was added to the cell pellet (in ~ 50 μl volume). After 15' of additional incubation at 37°C, three (HLA-DRB1*11:01) or five μl (HLA-DRB1*03:01) tetramers were added to 2/4 tubes. Tetramers were centrifuged at 14,000 rpm at 4C before use. Incubation with tetramers was performed for 2h at 37°C as per the manufacturer instructions. After incubation, the cells were washed again and two mixtures of fluorochrome-bound antibodies (see above) were added to panel A and panel B tubes respectively. After an additional wash, the cells were resuspended in 300 μl washing buffer and analysed through a Beckman Coulter Navios flow cytometer.

T-cell activation assays

PBMC from patients with SLE (n=15) and HC (n=5) who had been previously genotyped for HLA-DRB1 and studied for antigen specific T-cell phenotype at resting state were thawed, washed and resuspended in culture medium as described above and placed in a 48-well plate containing approximately 1x 10^6 cells in 500 μl culture medium per well. After one-hour recovery at 37°C, 5% CO_2, 20 μg/ml of penicilloylated-albumin peptide, histone-derived or EBV-derived peptides were added into separate wells according to subject genotype and antigen-specific T cell phenotypes at previous analyses (Azoury et al., 2018, Kalluri, Grummel et al., 2018, Kattah et al., 2015, Monneaux, Briand et al., 2000, Snir, Backlund et al., 2012). HC were tested for all three types of peptides independent on the result of antigen-specific T cell assays. Unstimulated cells and cells stimulated with 1μl/ml phytohaemagglutinin (PHA) served as negative and positive controls for each subject. An incubation time of 24h was set based on the average release kinetics of target cytokines according to the literature (**Appendix 11**) and on the kinetics of expression of surface markers of activation (Testi, Phillips et al., 1989). In addition, longer incubation times were excluded to minimise the risk of tolerance induction (Zhang et al., 2013).

After 24h-incubation, 100 μl of cell supernatant were collected from each well, purified from cell debris through centrifugation at 1500 rpm per one minute and stocked at -80°C until use. After thawing, cell supernatants were analysed for the expression of

the following T-helper cell-related cytokines through a multiplex bead array (Biolegend Legendplex, Cat. #741028): IL2, IL4, IL5, IL6, IL9, IL10, IL13, IL17A, IL17F, IFNγ, TNFα, IL22. All steps were performed in polypropylene tubes, all filled with 25 µl Assay buffer provided by the manufacturer. For each experimental session eight tubes were employed for a standard curve, encompassing six serial 1:4 dilutions of a Standard cocktail provided by the manufacturer into Assay buffer, one undiluted standard sample and a blank control. Twenty-five µl of undiluted supernatants were placed in each sample tube. After intense vortexing, 25 µl of cytokine binding beads were added to each sample or standard tube. Tubes were incubated with continuous basculation for two hours, protected from light. Eventually, 25 µl biotinylated antibodies were added to each tube, followed by an additional hour of incubation and mixing. Twenty-five µl of streptavidin-PE were then added to each tube, followed by 30 minutes of incubation and mixing. Sample and standard tubes were then washed with 200µl washing buffer and centrifuged at 1,000 g for 5'. After gentle aspiration of the washing buffer, samples and standard were resuspended in 200µl washing buffer. Fluorescence intensities were then measured through a Beckman Coulter Navios flow cytometer and converted into concentrations through a dedicated software provided by Biolegend.

Peptide-, PHA-stimulated and unstimulated cells from patients and controls were transferred into polypropylene tubes and washed with 2 ml washing solution per tube. Vital staining was then performed with Zombie Aqua™. After 10-minute incubation at room temperature and further washing, staining with panel C antibodies was performed. One duplicate per subject of PHA-stimulated cells were stained with anti-CD3, anti-CD4 and anti-CD8 antibodies only as described above. After 10 minutes of incubation at room temperature and further washing the cells were resuspended in 300 µl washing buffer and analysed through a Beckman Coulter Navios flow cytometer.

Activation marker and cytokine responses after stimulation with peptides and PHA were calculated as the fold change of each analyte compared to the negative (unstimulated control). Patients showing activation marker or cytokine variations exceeding one fold change for an analyte with respect to the negative control were classified as responders to that analyte.

Statistical analyses

Incidence rates of events-of-interest were calculated as the fraction of total events over the sum of the time intervals of observation of each subject. Cut-off values for optimal discrimination of anti-DNA-positive and beta-lactam allergic subjects based on histone-specific and penicilloylated albumin-specific T cell counts respectively were identified through receiver operating characteristics curves (**Appendix 7**). Since incomplete information on EBV status was available, the cut-off for EBV-specific T cells was set on the median value of EBV-specific T cells among subjects with established EBV infection. Frequencies of categorical variables among groups were compared by using the chi-square test with Fisher's exact correction and are expressed as percentages unless otherwise specified. Deviations from expected distributions of events among different analyses or in comparison with the general population were performed with the binomial test of hypotheses. Non-normally distributed continuous variables were compared through the Mann-Whitney's test or the Kruskall-Wallis' test among two or more groups. Variations in quantitative variables within the same subjects observed under different conditions were tested through the Wilcoxon's matched-pairs signed-rank test. Comparisons of normally distributed continuous variables among two or more groups were performed with the Student's t-test or with ANOVA with Bonferroni's correction respectively. Univariate Cox's regression analysis was used to analyse the association of disease phenotypes with time-dependent outcomes.

All analyses were performed with Statacorp STATA® version 15.0, Microsoft Excel® 2019 version or the OpenEpi online suite (http://www.openepi.com). Data are expressed as median (interquartile range, IQR) unless otherwise specified.

REFERENCES

Abdirama D, Tesch S, Griessbach AS, von Spee-Mayer C, Humrich JY, Stervbo U, Babel N, Meisel C, Alexander T, Biesen R, Bacher P, Scheffold A, Eckardt KU, Hiepe F, Radbruch A, Burmester GR, Riemekasten G, Enghard P (2021) Nuclear antigen-reactive CD4(+) T cells expand in active systemic lupus erythematosus, produce effector cytokines, and invade the kidneys. *Kidney Int* 99: 238-246

Abdulahad WH, Kallenberg CG, Limburg PC, Stegeman CA (2009) Urinary CD4+ effector memory T cells reflect renal disease activity in antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 60: 2830-8

Abdulahad WH, Stegeman CA, Limburg PC, Kallenberg CG (2008) Skewed distribution of Th17 lymphocytes in patients with Wegener's granulomatosis in remission. *Arthritis Rheum* 58: 2196-205

Abdulahad WH, Stegeman CA, van der Geld YM, Doornbos-van der Meer B, Limburg PC, Kallenberg CG (2007) Functional defect of circulating regulatory CD4+ T cells in patients with Wegener's granulomatosis in remission. *Arthritis Rheum* 56: 2080-91

Abrahamowicz M, Esdaile JM, Ramsey-Goldman R, Simon LS, Strand V, Lipsky PE (2018) Development and Validation of a Novel Evidence-Based Lupus Multivariable Outcome Score for Clinical Trials. *Arthritis & rheumatology* 70: 1450-1458

Aceves-Avila FJ, Benites-Godinez V (2008) Drug allergies may be more frequent in systemic lupus erythematosus than in rheumatoid arthritis. *J Clin Rheumatol* 14: 261-3

Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, Sallusto F, Napolitani G (2007) Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol* 8: 639-46

ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature (1999) The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 42: 599-608

Adam J, Pichler WJ, Yerly D (2011) Delayed drug hypersensitivity: models of T-cell stimulation. *Br J Clin Pharmacol* 71: 701-7

Agmon-Levin N, Mosca M, Petri M, Shoenfeld Y (2012) Systemic lupus erythematosus one disease or many? *Autoimmunity reviews* 11: 593-5

Ainiala H, Loukkola J, Peltola J, Korpela M, Hietaharju A (2001) The prevalence of neuropsychiatric syndromes in systemic lupus erythematosus. *Neurology* 57: 496-500

Alberici F, Martorana D, Bonatti F, Gioffredi A, Lyons PA, Vaglio A (2014) Genetics of ANCA-associated vasculitides: HLA and beyond. *Clin Exp Rheumatol* 32: S90-7

Allison AC, Eugui EM (2005) Mechanisms of action of mycophenolate mofetil in preventing acute and chronic allograft rejection. *Transplantation* 80: S181-90

Amanzadeh A, Amirzargar AA, Mohseni N, Arjang Z, Aghamohammadi A, Shokrgozar MA, Shokri F (2012) Association of HLA-DRB1, DQA1 and DQB1 Alleles and Haplotypes with Common Variable Immunodeficiency in Iranian Patients. *Avicenna J Med Biotechnol* 4: 103-12

Ambrose N, Morgan TA, Galloway J, Ionnoau Y, Beresford MW, Isenberg DA, Group UJS (2016) Differences in disease phenotype and severity in SLE across age groups. *Lupus* 25: 1542-1550

AMRER (Associazione Malati Reumatici Emilia-Romagna) (2015) Malattie reumatiche: presentato il primo 'censimento ufficiale' basato sulle esenzioni-ticket. In Il Notiziario del Malato reumatico,

Arango MT, Perricone C, Kivity S, Cipriano E, Ceccarelli F, Valesini G, Shoenfeld Y (2017) HLA-DRB1 the notorious gene in the mosaic of autoimmunity. *Immunol Res* 65: 82-98

Archila LD, DeLong JH, Wambre E, James EA, Robinson DM, Kwok WW (2014) Grass-specific CD4(+) T-cells exhibit varying degrees of cross-reactivity, implications for allergen-specific immunotherapy. *Clin Exp Allergy* 44: 986-98

Archila LD, Jeong D, Pascal M, Bartra J, Juan M, Robinson D, Farrington ML, Kwok WW (2015) Jug r 2-reactive CD4(+) T cells have a dominant immune role in walnut allergy. *J Allergy Clin Immunol* 136: 983-92 e7

Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, Smolen JS, Wofsy D, Boumpas DT, Kamen DL, Jayne D, Cervera R, Costedoat-Chalumeau N, Diamond B, Gladman DD, Hahn B, Hiepe F, Jacobsen S, Khanna D, Lerstrom K et al. (2019) 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis* 78: 1151-1159

Arnaud L, Tektonidou MG (2020) Long-term outcomes in systemic lupus erythematosus: trends over time and major contributors. *Rheumatology (Oxford)* 59: v29-v38

Artim-Esen B, Sahin S, Cene E, Sahinkaya Y, Barut K, Adrovic A, Ozluk Y, Kilicaslan I, Omma A, Gul A, Ocal L, Kasapcopur O, Inanc M (2017) Comparison of Disease Characteristics, Organ Damage, and Survival in Patients with Juvenile-onset and Adult-onset Systemic Lupus Erythematosus in a Combined Cohort from 2 Tertiary Centers in Turkey. *J Rheumatol* 44: 619-625

Atta AM, Santiago MB, Guerra FG, Pereira MM, Sousa Atta ML (2010) Autoimmune response of IgE antibodies to cellular self-antigens in systemic Lupus Erythematosus. *Int Arch Allergy Immunol* 152: 401-6

Azizi G, Ghanavatinejad A, Abolhassani H, Yazdani R, Rezaei N, Mirshafiey A, Aghamohammadi A (2016) Autoimmunity in primary T-cell immunodeficiencies. *Expert Rev Clin Immunol* 12: 989-1006

Azoury ME, Fili L, Bechara R, Scornet N, de Chaisemartin L, Weaver RJ, Claude N, Maillere B, Parronchi P, Joseph D, Pallardy M (2018) Identification of T-cell epitopes from benzylpenicillin conjugated to human serum albumin and implication in penicillin allergy. *Allergy* 73: 1662-1672

Bajema IM, Wilhelmus S, Alpers CE, Bruijn JA, Colvin RB, Cook HT, D'Agati VD, Ferrario F, Haas M, Jennette JC, Joh K, Nast CC, Noel LH, Rijnink EC, Roberts ISD, Seshan SV, Sethi S, Fogo AB (2018) Revision of the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis: clarification of definitions, and modified National Institutes of Health activity and chronicity indices. *Kidney Int* 93: 789-796

Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, Cepika AM, Acs P, Turner J, Anguiano E, Vinod P, Khan S, Obermoser G, Blankenship D, Wakeland E, Nassi L, Gotte A, Punaro M, Liu YJ, Banchereau J et al. (2016) Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. *Cell* 165: 1548-1550

Bang SY, Choi JY, Park S, Choi J, Hong SJ, Lee HS, Choi CB, Bae SC (2016) Brief Report: Influence of HLA-DRB1 Susceptibility Alleles on the Clinical Subphenotypes of Systemic Lupus Erythematosus in Koreans. *Arthritis & rheumatology* 68: 1190-6

Baqai T, Isenberg DA, Ioannou Y (2014) Pathogenesis of neurocognitive and neuropsychiatric manifestations in childhood-onset lupus: an overview. *Rheumatology Reports* 5: 6

Baragetti A, Ramirez G, Magnoni M, Garlaschelli K, Grigore L, Berteotti M, Scotti I, Bozzolo E, Berti A, Camici P (2017) Disease Trends Over Time and CD4+ CCR5+ T-Cells Expansion Predict Carotid Atherosclerosis Development in Patients with Systemic Lupus Erythematosus. *Nutrition, Metabolism and Cardiovascular Diseases*

Barata LT, Ying S, Meng Q, Barkansa J, Rajakulasingam K, Durham SR, Kay AB (1998) IL-4–and IL-5–positive T lymphocytes, eosinophils, and mast cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Allergy Clin Immunol* 101: 222-230

Barber MRW, Clarke AE (2017) Socioeconomic consequences of systemic lupus erythematosus. *Curr Opin Rheumatol* 29: 480-485

Barbhaiya M, Levine JM, Bykerk VP, Jannat-Khah D, Mandl LA (2021) Systemic rheumatic disease flares after SARS-CoV-2 vaccination among rheumatology outpatients in New York City. *Ann Rheum Dis*

Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, Dorgham K, Philippot Q, Rosain J, Beziat V, Manry J, Shaw E, Haljasmagi L, Peterson P, Lorenzo L, Bizien L, Trouillet-Assant S, Dobbs K, de Jesus AA, Belot A et al. (2020) Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 370

Bave U, Magnusson M, Eloranta ML, Perers A, Alm GV, Ronnblom L (2003) Fc gamma RIIa is expressed on natural IFN-alpha-producing cells (plasmacytoid dendritic cells) and is required for the IFN-alpha production induced by apoptotic cells combined with lupus IgG. *J Immunol* 171: 3296-302

Becattini S, Latorre D, Mele F, Foglierini M, De Gregorio C, Cassotta A, Fernandez B, Kelderman S, Schumacher TN, Corti D, Lanzavecchia A, Sallusto F (2015) T cell immunity. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines. *Science* 347: 400-6

Beheshti SA, Shamsasenjan K, Ahmadi M, Abbasi B (2022) CAR Treg: A new approach in the treatment of autoimmune diseases. *Int Immunopharmacol* 102: 108409

Benagiano M, Borghi MO, Romagnoli J, Mahler M, Della Bella C, Grassi A, Capitani N, Emmi G, Troilo A, Silvestri E, Emmi L, Alnwaisri H, Bitetti J, Tapinassi S, Prisco D, Baldari CT, Meroni PL, D'Elios MM (2019) Interleukin-17/Interleukin-21 and Interferon-g producing T cells specific for β2

Glycoprotein I in atherosclerosis inflammation of systemic lupus erythematosus

patients with antiphospholipid syndrome. *Haematologica*

Bentham J, Morris DL, Graham DSC, Pinder CL, Tombleson P, Behrens TW, Martin J, Fairfax BP, Knight JC, Chen L, Replogle J, Syvanen AC, Ronnblom L, Graham RR, Wither JE, Rioux JD, Alarcon-Riquelme ME, Vyse TJ (2015) Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat Genet* 47: 1457-1464

Benucci M, Del Rosso A, Li Gobbi F, Manfredi M, Cerinic MM, Salvarani C (2005) Systemic lupus erythematosus (SLE) in Italy: an Italian prevalence study based on a two-step strategy in an area of Florence (Scandicci-Le Signe). *Med Sci Monit* 11: CR420-5

Berner BR, Tary-Lehmann M, Yonkers NL, Askari AD, Lehmann PV, Anthony DD (2005) Phenotypic and functional analysis of EBV-specific memory CD8 cells in SLE. *Cell Immunol* 235: 29-38

Bharadwaj M, Illing P, Theodossis A, Purcell AW, Rossjohn J, McCluskey J (2012) Drug hypersensitivity and human leukocyte antigens of the major histocompatibility complex. *Annu Rev Pharmacol Toxicol* 52: 401-31

Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH (1992) Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 35: 630-40

Bonacchi R, Rocca MA, Ramirez GA, Bozzolo EP, Canti V, Preziosa P, Valsasina P, Riccitelli GC, Meani A, Moiola L, Rovere-Querini P, Manfredi AA, Filippi M (2020) Resting state network functional connectivity abnormalities in systemic lupus erythematosus: correlations with neuropsychiatric impairment. *Mol Psychiatry*

Bonelli M, Goschl L, Bluml S, Karonitsch T, Steiner CW, Steiner G, Smolen JS, Scheinecker C (2014) CD4(+)CD25(-)Foxp3(+) T cells: a marker for lupus nephritis? *Arthritis Res Ther* 16: R104

Bonelli M, von Dalwigk K, Savitskaya A, Smolen JS, Scheinecker C (2008) Foxp3 expression in CD4+ T cells of patients with systemic lupus erythematosus: a comparative phenotypic analysis. *Ann Rheum Dis* 67: 664-71

Boonpiyathad T, Sokolowska M, Morita H, Ruckert B, Kast JI, Wawrzyniak M, Sangasapaviliya A, Pradubpongsa P, Fuengthong R, Thantiworasit P, Sirivichayakul S, Kwok WW, Ruxrungtham K, Akdis M, Akdis CA (2019) Der p 1-specific regulatory T-cell response during house dust mite allergen immunotherapy. *Allergy* 74: 976-985

Bortoluzzi A, Fanouriakis A, Appenzeller S, Costallat L, Scire CA, Murphy E, Bertsias G, Hanly J, Govoni M (2017) Validity of the Italian algorithm for the attribution of neuropsychiatric events in systemic lupus erythematosus: a retrospective multicentre international diagnostic cohort study. *BMJ Open* 7: e015546

Bortoluzzi A, Scire CA, Bombardieri S, Caniatti L, Conti F, De Vita S, Doria A, Ferraccioli G, Gremese E, Mansutti E, Mathieu A, Mosca M, Padovan M, Piga M, Tincani A, Tola MR, Tomietto P, Valesini G, Zen M, Govoni M et al. (2015) Development and validation of a new algorithm for attribution of neuropsychiatric events in systemic lupus erythematosus. *Rheumatology (Oxford)* 54: 891-8

Bosch X, Guilabert A, Pallares L, Cerveral R, Ramos-Casals M, Bove A, Ingelmo M, Font J (2006) Infections in systemic lupus erythematosus: a prospective and controlled study of 110 patients. *Lupus* 15: 584-9

Brack A, Geisler A, Martinez-Taboada VM, Younge BR, Goronzy JJ, Weyand CM (1997) Giant cell vasculitis is a T cell-dependent disease. *Mol Med* 3: 530-43

Brown EE, Edberg JC, Kimberly RP (2007) Fc receptor genes and the systemic lupus erythematosus diathesis. *Autoimmunity* 40: 567-81

Bruce IN, Urowitz M, van Vollenhoven R, Aranow C, Fettiplace J, Oldham M, Wilson B, Molta C, Roth D, Gordon D (2016) Long-term organ damage accrual and safety in patients with SLE treated with belimumab plus standard of care. *Lupus* 25: 699-709

Bruns A, Blass S, Hausdorf G, Burmester GR, Hiepe F (2000) Nucleosomes are major T and B cell autoantigens in systemic lupus erythematosus. *Arthritis Rheum* 43: 2307-15

Bruschi M, Moroni G, Sinico RA, Franceschini F, Fredi M, Vaglio A, Cavagna L, Petretto A, Pratesi F, Migliorini P, Locatelli F, Pazzola G, Pesce G, Bagnasco M, Manfredi A, Ramirez GA, Esposito P, Murdaca G, Negrini S, Cipriani L et al. (2021) Serum IgG2 antibody multicomposition in systemic lupus erythematosus and lupus nephritis (Part 1): cross-sectional analysis. *Rheumatology (Oxford)* 60: 3176-3188

Burns LA, Maroof A, Marshall D, Steel KJA, Lalnunhlimi S, Cole S, Catrina A, Kirkham B, Taams LS (2020) Presence, function, and regulation of IL-17F-expressing human CD4(+) T cells. *Eur J Immunol* 50: 568-580

Carli L, Tani C, Vagnani S, Signorini V, Mosca M (2015) Leukopenia, lymphopenia, and neutropenia in systemic lupus erythematosus: Prevalence and clinical impact--A systematic literature review. *Semin Arthritis Rheum* 45: 190-4

Carmona FD, Coit P, Saruhan-Direskeneli G, Hernandez-Rodriguez J, Cid MC, Solans R, Castaneda S, Vaglio A, Direskeneli H, Merkel PA, Boiardi L, Salvarani C, Gonzalez-Gay MA, Martin J, Sawalha AH, Spanish GCASG, Italian GCASG, Turkish Takayasu Study G, Vasculitis Clinical Research C (2017) Analysis of the common genetic component of large-vessel vasculitides through a meta-Immunochip strategy. *Sci Rep* 7: 43953

Carter LM, Isenberg DA, Ehrenstein MR (2013) Elevated Serum BAFF Levels Are Associated With Rising Anti–Double-Stranded DNA Antibody Levels and Disease Flare Following B Cell Depletion Therapy in Systemic Lupus Erythematosus. *Arthritis Rheum* 65: 2672-2679

Castrejon I, Tani C, Jolly M, Huang A, Mosca M (2014) Indices to assess patients with systemic lupus erythematosus in clinical trials, long-term observational studies, and clinical care. *Clin Exp Rheumatol* 32: S-85-95

Ceccarelli F, Perricone C, Borgiani P, Ciccacci C, Rufini S, Cipriano E, Alessandri C, Spinelli FR, Sili Scavalli A, Novelli G, Valesini G, Conti F (2015) Genetic Factors in Systemic Lupus Erythematosus: Contribution to Disease Phenotype. *Journal of immunology research* 2015: 745647

Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramon E, Buonaiuto V, Jacobsen S, Zeher MM, Tarr T, Tincani A, Taglietti M, Theodossiades G, Nomikou E, Galeazzi M, Bellisai F, Meroni PL, Derksen RH, de Groot PG, Baleva M et al. (2015) Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. *Ann Rheum Dis* 74: 1011-8

Chakravarty EF, Michaud K, Katz R, Wolfe F (2013) Increased incidence of herpes zoster among patients with systemic lupus erythematosus. *Lupus* 22: 238-44

Chang CC, Chang YS, Chen WS, Chen YH, Chen JH (2016) Effects of annual influenza vaccination on morbidity and mortality in patients with Systemic Lupus Erythematosus: A Nationwide Cohort Study. *Sci Rep* 6: 37817

Charles N, Hardwick D, Daugas E, Illei GG, Rivera J (2010) Basophils and the T helper 2 environment can promote the development of lupus nephritis. *Nat Med* 16: 701-7

Chen L, Morris DL, Vyse TJ (2017) Genetic advances in systemic lupus erythematosus: an update. *Curr Opin Rheumatol* 29: 423-433

Chen L, Wang YF, Liu L, Bielowka A, Ahmed R, Zhang H, Tombleson P, Roberts AL, Odhams CA, Cunninghame Graham DS, Zhang X, Yang W, Vyse TJ, Morris DL (2020) Genome-wide assessment of genetic risk for systemic lupus erythematosus and disease severity. *Hum Mol Genet* 29: 1745-1756

Chen M, Daha MR, Kallenberg CG (2010) The complement system in systemic autoimmune disease. *J Autoimmun* 34: J276-86

Chighizola CB, Raschi E, Borghi MO, Meroni PL (2015) Update on the pathogenesis and treatment of the antiphospholipid syndrome. *Curr Opin Rheumatol* 27: 476-82

Chowdhury B, Tsokos CG, Krishnan S, Robertson J, Fisher CU, Warke RG, Warke VG, Nambiar MP, Tsokos GC (2005) Decreased stability and translation of T cell receptor zeta mRNA with an alternatively spliced 3'-untranslated region contribute to zeta chain down-regulation in patients with systemic lupus erythematosus. *J Biol Chem* 280: 18959-66

Chung SA, Brown EE, Williams AH, Ramos PS, Berthier CC, Bhangale T, Alarcon-Riquelme ME, Behrens TW, Criswell LA, Graham DC, Demirci FY, Edberg JC, Gaffney PM, Harley JB, Jacob CO, Kamboh MI, Kelly JA, Manzi S, Moser-Sivils KL, Russell LP et al. (2014) Lupus Nephritis Susceptibility Loci in Women with Systemic Lupus Erythematosus. *J Am Soc Nephrol*

Cianciotti BC, Ruggiero E, Campochiaro C, Oliveira G, Magnani ZI, Baldini M, Doglio M, Tassara M, Manfredi AA, Baldissera E, Ciceri F, Cieri N, Bonini C (2019) CD4(+) memory stem T cells recognizing citrullinated epitopes are expanded in patients with Rheumatoid Arthritis and sensitive to TNF-alpha blockade. *Arthritis & rheumatology*

Cianciotti BC, Ruggiero E, Campochiaro C, Oliveira G, Magnani ZI, Baldini M, Doglio M, Tassara M, Manfredi AA, Baldissera E, Ciceri F, Cieri N, Bonini C (2020) CD4+ Memory Stem T Cells Recognizing Citrullinated Epitopes Are Expanded in Patients With Rheumatoid Arthritis and Sensitive to Tumor Necrosis Factor Blockade. *Arthritis & rheumatology* 72: 565-575

Ciceri F, Beretta L, Scandroglio AM, Colombo S, Landoni G, Ruggeri A, Peccatori J, D'Angelo A, De Cobelli F, Rovere-Querini P, Tresoldi M, Dagna L, Zangrillo A (2020) Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome

(MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. *Crit Care Resusc* 22: 95-97

Cieri N, Oliveira G, Greco R, Forcato M, Taccioli C, Cianciotti B, Valtolina V, Noviello M, Vago L, Bondanza A, Lunghi F, Marktel S, Bellio L, Bordignon C, Bicciato S, Peccatori J, Ciceri F, Bonini C (2015) Generation of human memory stem T cells after haploidentical T-replete hematopoietic stem cell transplantation. *Blood* 125: 2865-74

Cleanthous S, Tyagi M, Isenberg DA, Newman SP (2012) What do we know about self-reported fatigue in systemic lupus erythematosus? *Lupus* 21: 465-76

Clemente-Casares X, Blanco J, Ambalavanan P, Yamanouchi J, Singha S, Fandos C, Tsai S, Wang J, Garabatos N, Izquierdo C, Agrawal S, Keough MB, Yong VW, James E, Moore A, Yang Y, Stratmann T, Serra P, Santamaria P (2016) Expanding antigen-specific regulatory networks to treat autoimmunity. *Nature* 530: 434-40

Conti F, Ceccarelli F, Perricone C, Massaro L, Marocchi E, Miranda F, Spinelli FR, Truglia S, Alessandri C, Valesini G (2015) Systemic Lupus Erythematosus with and without Anti-dsDNA Antibodies: Analysis from a Large Monocentric Cohort. *Mediators Inflamm* 2015: 328078

Contin-Bordes C, Lazaro E, Richez C, Jacquemin C, Caubet O, Douchet I, Viallard JF, Moreau JF, Pellegrin JL, Blanco P (2011) Expansion of myelin autoreactive CD8+ T lymphocytes in patients with neuropsychiatric systemic lupus erythematosus. *Ann Rheum Dis* 70: 868-71

Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS (2002) Risk factors for development of systemic lupus erythematosus: allergies, infections, and family history. *J Clin Epidemiol* 55: 982-9

Cortes Verdu R, Pego-Reigosa JM, Seoane-Mato D, Morcillo Valle M, Palma Sanchez D, Moreno Martinez MJ, Mayor Gonzalez M, Atxotegi Saenz de Buruaga J, Urionaguena Onaindia I, Blanco Caceres BA, Silva-Fernandez L, Sivera F, Blanco FJ, Sanchez-Piedra C, Diaz-Gonzalez F, Bustabad S (2020) Prevalence of systemic lupus erythematosus in Spain: higher than previously reported in other countries? *Rheumatology (Oxford)*

Costedoat-Chalumeau N, Dunogue B, Morel N, Le Guern V, Guettrot-Imbert G (2014) Hydroxychloroquine: a multifaceted treatment in lupus. *Presse Med* 43: e167-80

Crispin JC, Tsokos GC (2015) Pathogenesis of Lupus. In *Rheumatology, VI edition*, Mosby

Cruz GI, Shao X, Quach H, Ho KA, Sterba K, Noble JA, Patsopoulos NA, Busch MP, Triulzi DJ, Wong WS, Solomon BD, Niederhuber JE, Criswell LA, Barcellos LF (2016) A Child's HLA-DRB1 genotype increases maternal risk of systemic lupus erythematosus. *J Autoimmun* 74: 201-207

Cui YL, Zhou BY, Gao GC (2021) A systematic review and meta-analysis of the correlation between Helicobacter pylori infection and chronic urticaria. *Ann Palliat Med* 10: 10584-10590

Danchenko N, Satia JA, Anthony MS (2006) Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus* 15: 308-18

Danza A, Ruiz-Irastorza G (2013) Infection risk in systemic lupus erythematosus patients: susceptibility factors and preventive strategies. *Lupus* 22: 1286-94

de Boer BA, Fillié YE, Kruize YC, Yazdanbakhsh M (1998) Antigen-stimulated IL-4, IL-13 and IFN-γ production by human T cells at a single-cell level. *Eur J Immunol* 28: 3154-3160

de Holanda MI, Klumb E, Imada A, Lima LA, Alcantara I, Gregorio F, Christiani LF, Martins CO, Timoner BE, Motta J, Pozzan R, Porto LC (2018) The prevalence of HLA alleles in a lupus nephritis population. *Transpl Immunol* 47: 37-43

de Moerloose P, Fickentscher C, Boehlen F, Tiercy JM, Kruithof EKO, Brandt KJ (2017) Patient-derived anti-beta2GP1 antibodies recognize a peptide motif pattern and not a specific sequence of residues. *Haematologica* 102: 1324-1332

De Re V, Caggiari L, Monti G, Libra M, Spina M, Dolcetti R, De Zorzi M, Racanelli V, Crovatto M, Toffoli G (2010) HLA DR-DQ combination associated with the increased risk of developing human HCV positive non-Hodgkin's lymphoma is related to the type II mixed cryoglobulinemia. *Tissue Antigens* 75: 127-35

Dehlink E, Gruber S, Eiwegger T, Gruber D, Mueller T, Huber WD, Klepetko W, Rumpold H, Urbanek R, Szepfalusi Z (2006) Immunosuppressive therapy does not prevent the occurrence of immunoglobulin E-mediated allergies in children and adolescents with organ transplants. *Pediatrics* 118: e764-70

Dema B, Charles N (2014) Advances in mechanisms of systemic lupus erythematosus. *Discov Med* 17: 247-55

Dema B, Lamri Y, Pellefigues C, Pacreau E, Saidoune F, Bidault C, Karasuyama H, Sacre K, Daugas E, Charles N (2017) Basophils contribute to pristane-induced Lupus-like nephritis model. *Sci Rep* 7: 7969

Dema B, Pellefigues C, Hasni S, Gault N, Jiang C, Ricks TK, Bonelli MM, Scheffel J, Sacre K, Jablonski M, Gobert D, Papo T, Daugas E, Illei G, Charles N, Rivera J (2014) Autoreactive IgE is prevalent in systemic lupus erythematosus and is associated with increased disease activity and nephritis. *PLoS One* 9: e90424

Deng J, Younge BR, Olshen RA, Goronzy JJ, Weyand CM (2010) Th17 and Th1 T-cell responses in giant cell arteritis. *Circulation* 121: 906-15

Deng Y, Tsao BP (2010) Genetic susceptibility to systemic lupus erythematosus in the genomic era. *Nat Rev Rheumatol* 6: 683-92

Di Matteo A, Filippucci E, Cipolletta E, Satulu I, Hurnakova J, Lato V, De Angelis R, Horvath R, Pavelka K, Salaffi F, Grassi W (2018) Entheseal involvement in patients with systemic lupus erythematosus: an ultrasound study. *Rheumatology (Oxford)* 57: 1822-1829

Di Matteo A, Smerilli G, Cipolletta E, Salaffi F, De Angelis R, Di Carlo M, Filippucci E, Grassi W (2021) Imaging of Joint and Soft Tissue Involvement in Systemic Lupus Erythematosus. *Curr Rheumatol Rep* 23: 73

Diao L, Tao J, Wang Y, Hu Y, He W (2019) Co-Delivery Of Dihydroartemisinin And HMGB1 siRNA By TAT-Modified Cationic Liposomes Through The TLR4 Signaling Pathway For Treatment Of Lupus Nephritis. *Int J Nanomedicine* 14: 8627-8645

Diaz-Gallo LM, Oke V, Lundstrom E, Elvin K, Ling Wu Y, Eketjall S, Zickert A, Gustafsson JT, Jonsen A, Leonard D, Birmingham DJ, Nordmark G, Bengtsson AA, Ronnblom L, Gunnarsson I, Yu CY, Padyukov L, Svenungsson E (2021) Four Systemic Lupus Erythematosus Subgroups, Defined by Autoantibodies Status, Differ Regarding HLA-DRB1 Genotype Associations and Immunological and Clinical Manifestations. *ACR Open Rheumatol*

Dieker J, Tel J, Pieterse E, Thielen A, Rother N, Bakker M, Fransen J, Dijkman HB, Berden JH, de Vries JM, Hilbrands LB, van der Vlag J (2016) Circulating Apoptotic Microparticles in Systemic Lupus Erythematosus Patients Drive the Activation of Dendritic Cell Subsets and Prime Neutrophils for NETosis. *Arthritis & rheumatology* 68: 462-72

Diumenjo MS, Lisanti M, Valles R, Rivero I (1985) [Allergic manifestations of systemic lupus erythematosus]. *Allergol Immunopathol (Madr)* 13: 323-6

Dolff S, Abdulahad WH, van Dijk MC, Limburg PC, Kallenberg CG, Bijl M (2010) Urinary T cells in active lupus nephritis show an effector memory phenotype. *Ann Rheum Dis* 69: 2034-41

Dolton G, Tungatt K, Lloyd A, Bianchi V, Theaker SM, Trimby A, Holland CJ, Donia M, Godkin AJ, Cole DK, Straten PT, Peakman M, Svane IM, Sewell AK (2015) More tricks with tetramers: a practical guide to staining T cells with peptide-MHC multimers. *Immunology* 146: 11-22

Dong RP, Kimura A, Numano F, Nishimura Y, Sasazuki T (1992) HLA-linked susceptibility and resistance to Takayasu arteritis. *Heart Vessels Suppl* 7: 73-80

Doria A, Gatto M, Iaccarino L, Punzi L (2015) Value and goals of treat-to-target in systemic lupus erythematosus: knowledge and foresight. *Lupus* 24: 507-15

Dorner T (2010) Therapy: Hydroxychloroquine in SLE: old drug, new perspectives. *Nat Rev Rheumatol* 6: 10-1

dos Santos M, Bringhenti RN, Rodrigues PG, do Nascimento JF, Pereira SV, Zancan R, Monticielo OA, Gasparin AA, de Castro WP, Veronese FV (2015) Podocyte-associated mRNA profiles in kidney tissue and in urine of patients with active lupus nephritis. *Int J Clin Exp Pathol* 8: 4600-13

Draborg AH, Jacobsen S, Westergaard M, Mortensen S, Larsen JL, Houen G, Duus K (2014) Reduced response to Epstein-Barr virus antigens by T-cells in systemic lupus erythematosus patients. *Lupus science & medicine* 1: e000015

Draborg AH, Jorgensen JM, Muller H, Nielsen CT, Jacobsen S, Iversen LV, Theander E, Nielsen LP, Houen G, Duus K (2012) Epstein-Barr virus early antigen diffuse (EBV-EA/D)-directed immunoglobulin A antibodies in systemic lupus erythematosus patients. *Scand J Rheumatol* 41: 280-9

Draborg AH, Rasmussen NS, Larsen JL, Jorgensen CS, Sandhu N, Skogstrand K, Jacobsen S, Houen G (2018) Immune responses to an early lytic cytomegalovirus antigen in systemic lupus erythematosus patients: T-cell responses, cytokine secretions and antibody status. *PLoS One* 13: e0193244

Draborg AH, Sandhu N, Larsen N, Lisander Larsen J, Jacobsen S, Houen G (2016) Impaired Cytokine Responses to Epstein-Barr Virus Antigens in Systemic Lupus Erythematosus Patients. *Journal of immunology research* 2016: 6473204

Duffy KN, Duffy CM, Gladman DD (1991) Infection and disease activity in systemic lupus erythematosus: a review of hospitalized patients. *J Rheumatol* 18: 1180-4

Duhen T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F (2009) Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol* 10: 857-63

Emlen W, Niebur J, Kadera R (1994) Accelerated in vitro apoptosis of lymphocytes from patients with systemic lupus erythematosus. *J Immunol* 152: 3685-92

Engelmann B, Massberg S (2013) Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* 13: 34-45

Fanouriakis A, Kostopoulou M, Alunno A, Aringer M, Bajema I, Boletis JN, Cervera R, Doria A, Gordon C, Govoni M, Houssiau F, Jayne D, Kouloumas M, Kuhn A, Larsen JL, Lerstrom K, Moroni G, Mosca M, Schneider M, Smolen JS et al. (2019) 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. *Ann Rheum Dis* 78: 736-745

Fanouriakis A, Kostopoulou M, Cheema K, Anders HJ, Aringer M, Bajema I, Boletis J, Frangou E, Houssiau FA, Hollis J, Karras A, Marchiori F, Marks SD, Moroni G, Mosca M, Parodis I, Praga M, Schneider M, Smolen JS, Tesar V et al. (2020) 2019 Update of the Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of lupus nephritis. *Ann Rheum Dis* 79: 713-723

Faria R, Goncalves J, Dias R (2017) Neuropsychiatric Systemic Lupus Erythematosus Involvement: Towards a Tailored Approach to Our Patients? *Rambam Maimonides Med J* 8

Farinha F, Pepper RJ, Oliveira DG, McDonnell T, Isenberg DA, Rahman A (2020) Outcomes of membranous and proliferative lupus nephritis - analysis of a single-centre cohort with more than 30 years of follow-up. *Rheumatology (Oxford)* 59: 3314-3323

Fasano S, Coscia MA, Pierro L, Ciccia F (2021) Which patients with systemic lupus erythematosus in remission can withdraw low dose steroids? Results from a single inception cohort study. *Lupus* 30: 991-997

Fasano S, Pierro L, Pantano I, Iudici M, Valentini G (2017) Longterm Hydroxychloroquine Therapy and Low-dose Aspirin May Have an Additive Effectiveness in the Primary Prevention of Cardiovascular Events in Patients with Systemic Lupus Erythematosus. *J Rheumatol* 44: 1032-1038

Fayyaz A, Igoe A, Kurien BT, Danda D, James JA, Stafford HA, Scofield RH (2015) Haematological manifestations of lupus. *Lupus science & medicine* 2: e000078

Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A (2003) IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. *J Immunol* 170: 2106-12

Ferriani MP, Silva MF, Pereira RM, Terreri MT, Saad Magalhaes C, Bonfa E, Pastorino AC, Carolina Dos Santos M, Appenzeller S, Ferriani VP, Len CA, Sallum AM, Liborio J, Monteiro de Castro TC, Silva CA (2015) Chronic Spontaneous Urticaria: A Survey of 852 Cases of Childhood-Onset Systemic Lupus Erythematosus. *Int Arch Allergy Immunol* 167: 186-92

Fischetti F, Tedesco F (2006) Cross-talk between the complement system and endothelial cells in physiologic conditions and in vascular diseases. *Autoimmunity* 39: 417-28

Franklyn K, Lau CS, Navarra SV, Louthrenoo W, Lateef A, Hamijoyo L, Wahono CS, Chen SL, Jin O, Morton S, Hoi A, Huq M, Nikpour M, Morand EF, Asia-Pacific Lupus C (2016) Definition and initial validation of a Lupus Low Disease Activity State (LLDAS). *Ann Rheum Dis* 75: 1615-21

Free ME, Stember KG, Hess JJ, McInnis EA, Lardinois O, Hogan SL, Hu Y, Mendoza C, Le AK, Guseman AJ, Pilkinton MA, Bortone DS, Cowens K, Sidney J, Karosiene E, Peters B, James E, Kwok WW, Vincent BG, Mallal SA et al. (2019) Restricted myeloperoxidase epitopes drive the adaptive immune response in MPO-ANCA vasculitis. *J Autoimmun*: 102306

Fritsch RD, Shen X, Illei GG, Yarboro CH, Prussin C, Hathcock KS, Hodes RJ, Lipsky PE (2006) Abnormal differentiation of memory T cells in systemic lupus erythematosus. *Arthritis Rheum* 54: 2184-97

Funauchi M, Ikoma S, Enomoto H, Horiuchi A (1998) Decreased Th1-like and increased Th2-like cells in systemic lupus erythematosus. *Scand J Rheumatol* 27: 219-24

Furie R, Khamashta M, Merrill JT, Werth VP, Kalunian K, Brohawn P, Illei GG, Drappa J, Wang L, Yoo S, Investigators CDS (2017) Anifrolumab, an Anti-Interferon-alpha Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus. *Arthritis & rheumatology* 69: 376-386

Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzova D, Sanchez-Guerrero J, Schwarting A, Merrill JT, Chatham WW, Stohl W, Ginzler EM, Hough DR, Zhong ZJ, Freimuth W, van Vollenhoven RF, Group B-S (2011) A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum* 63: 3918-30

Furie RA, Morand EF, Bruce IN, Manzi S, Kalunian KC, Vital EM, Ford TL, Gupta R, Hiepe F, Santiago M (2019) Type I interferon inhibitor anifrolumab in active systemic lupus erythematosus (TULIP-1): a randomised, controlled, phase 3 trial. *The Lancet Rheumatology* 1: e208-e219

Garcia-Arellano S, Hernandez-Palma LA, Bucala R, Hernandez-Bello J, De la Cruz-Mosso U, Garcia-Iglesias T, Cerpa-Cruz S, Padilla-Gutierrez JR, Valle Y, Sonanez-Organis JG, Parra-Rojas I, Pereira-Suarez AL, Munoz-Valle JF (2018) Th1/Th17 Cytokine Profile is Induced by Macrophage Migration Inhibitory Factor in Peripheral Blood Mononuclear Cells from Rheumatoid Arthritis Patients. *Curr Mol Med* 18: 679-688

Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, Punaro M, Baisch J, Guiducci C, Coffman RL, Barrat FJ, Banchereau J, Pascual V (2011) Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci Transl Med* 3: 73ra20

Gaspar P, Cohen H, Isenberg DA (2020) The assessment of patients with the antiphospholipid antibody syndrome: where are we now? *Rheumatology (Oxford)* 59: 1489-1494

Gattinoni L, Speiser DE, Lichterfeld M, Bonini C (2017) T memory stem cells in health and disease. *Nat Med* 23: 18-27

Gavand PE, Serio I, Arnaud L, Costedoat-Chalumeau N, Carvelli J, Dossier A, Hinschberger O, Mouthon L, Le Guern V, Korganow AS, Poindron V, Gourguechon C, Lavigne C, Maurier F, Labro G, Heymonet M, Artifoni M, Viau AB, Deligny C, Sene T et al. (2017) Clinical spectrum and therapeutic management of systemic lupus

erythematosus-associated macrophage activation syndrome: A study of 103 episodes in 89 adult patients. *Autoimmunity reviews* 16: 743-749

Gerosa M, Ramirez G, Bellocchi C, Argolini L, Moroni L, Cornalba M, Farina N, Dagna L, Caporali R, Bozzolo E (2020) FRI0167 LONG TERM CLINICAL OUTCOME IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS FOLLOWED FOR MORE THAN 20 YEARS IN THREE ITALIAN TERTIARY REFERRAL CENTERS: THE MILAN SYSTEMIC LUPUS ERYTHEMATOSUS CONSORTIUM (SMILE) COHORT. In BMJ Publishing Group Ltd

Ghiggeri GM, D'Alessandro M, Bartolomeo D, Degl'Innocenti ML, Magnasco A, Lugani F, Prunotto M, Bruschi M (2019) An Update on Antibodies to Necleosome Components as Biomarkers of Sistemic Lupus Erythematosus and of Lupus Flares. *International journal of molecular sciences* 20

Gladman DD (2015) Toward Treating to Target in Psoriatic Arthritis. *J Rheumatol Suppl* 93: 14-6

Gladman DD, Goldsmith CH, Urowitz MB, Bacon P, Fortin P, Ginzler E, Gordon C, Hanly JG, Isenberg DA, Petri M, Nived O, Snaith M, Sturfelt G (2000) The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for Systemic Lupus Erythematosus International Comparison. *J Rheumatol* 27: 373-6

Gladman DD, Ibanez D, Urowitz MB (2002) Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 29: 288-91

Goldblatt F, Chambers S, Rahman A, Isenberg DA (2009) Serious infections in British patients with systemic lupus erythematosus: hospitalisations and mortality. *Lupus* 18: 682-9

Golder V, Kandane-Rathnayake R, Huq M, Nim HT, Louthrenoo W, Luo SF, Wu Y-JJ, Lateef A, Sockalingam S, Navarra SV, Zamora L, Hamijoyo L, Katsumata Y, Harigai M, Chan M, O'Neill S, Goldblatt F, Lau CS, Li ZG, Hoi A et al. (2019) Lupus low disease activity state as a treatment endpoint for systemic lupus erythematosus: a prospective validation study. *The Lancet Rheumatology* 1: e95-e102

Gonzalez-Figueroa P, Roco JA, Papa I, Nunez Villacis L, Stanley M, Linterman MA, Dent A, Canete PF, Vinuesa CG (2021) Follicular regulatory T cells produce neuritin to regulate B cells. *Cell* 184: 1775-1789 e19

Gonzalez-Galarza Faviel F, McCabe A, Santos Eduardo J Md, Jones J, Takeshita L, Ortega-Rivera Nestor D, Cid-Pavon Glenda MD, Ramsbottom K, Ghattaoraya G, Alfirevic A, Middleton D, Jones Andrew R (2019) Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools. *Nucleic Acids Res* 48: D783-D788

Gordon C, Amissah-Arthur MB, Gayed M, Brown S, Bruce IN, D'Cruz D, Empson B, Griffiths B, Jayne D, Khamashta M, Lightstone L, Norton P, Norton Y, Schreiber K, Isenberg D, British Society for Rheumatology Standards A, Guidelines Working G (2018) The British Society for Rheumatology guideline for the management of systemic lupus erythematosus in adults: Executive Summary. *Rheumatology (Oxford)* 57: 14-18

Govoni M, Bortoluzzi A, Padovan M, Silvagni E, Borrelli M, Donelli F, Ceruti S, Trotta F (2016) The diagnosis and clinical management of the neuropsychiatric manifestations of lupus. *J Autoimmun* 74: 41-72

Govoni M, Castellino G, Bosi S, Napoli N, Trotta F (2006) Incidence and prevalence of systemic lupus erythematosus in a district of north Italy. *Lupus* 15: 110-3

Grant CR, Liberal R, Mieli-Vergani G, Vergani D, Longhi MS (2015) Regulatory T-cells in autoimmune diseases: challenges, controversies and--yet--unanswered questions. *Autoimmunity reviews* 14: 105-16

Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY et al. (2020) Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 382: 1708-1720

Guo Y, Fang J, Ma J, Li G, Zhang L, He J, Xu L, Lai X, Yin W, Xiong Y, Liu L, Zhang Y, Pan G, Chen Z (2019) Correlation between use of immunosuppressive agents and transplant-acquired allergies in renal transplant recipients. *Transl Androl Urol* 8: 442-447

Gupta S, Nakabo S, Chu J, Hasni S, Kaplan MJ (2021a) Correspondence on 'Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine'. *Ann Rheum Dis*

Gupta S, Nakabo S, Chu J, Hasni S, Kaplan MJ (2021b) Correspondence on ‘Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic

lupus erythematosus under long-term treatment with hydroxychloroquine'. annrheumdis-2020-219648

Hamilton LA, Guarascio AJ (2019) Tetracycline Allergy. *Pharmacy (Basel)* 7

Hanly JG (1997) Disease activity, cumulative damage and quality of life in systematic lupus erythematosus: results of a cross-sectional study. *Lupus* 6: 243-7

Hanly JG (2014) Diagnosis and management of neuropsychiatric SLE. *Nat Rev Rheumatol* 10: 338-47

Harboe E, Greve OJ, Beyer M, Goransson LG, Tjensvoll AB, Maroni S, Omdal R (2008) Fatigue is associated with cerebral white matter hyperintensities in patients with systemic lupus erythematosus. *J Neurol Neurosurg Psychiatry* 79: 199-201

Hartmann BM, Marjanovic N, Nudelman G, Moran TM, Sealfon SC (2014) Combinatorial cytokine code generates anti-viral state in dendritic cells. *Front Immunol* 5: 73

Hasni S, Gupta S, Davis M, Poncio E, Temesgen-Oyelakin Y, Joyal E, Fike A, Manna Z, Auh S, Shi Y, Chan D, Carlucci P, Biehl A, Dema B, Charles N, Balow JE, Waldman M, Siegel RM, Kaplan MJ, Rivera J (2019) Safety and Tolerability of Omalizumab: A Randomized Clinical Trial of Humanized Anti-IgE Monoclonal Antibody in Systemic Lupus Erythematosus. *Arthritis & rheumatology* 71: 1135-1140

He J, Lang G, Ding S, Li L (2013) Pathological role of interleukin-17 in poly I:C-induced hepatitis. *PLoS One* 8: e73909

Henault J, Riggs JM, Karnell JL, Liarski VM, Li J, Shirinian L, Xu L, Casey KA, Smith MA, Khatry DB, Izhak L, Clarke L, Herbst R, Ettinger R, Petri M, Clark MR, Mustelin T, Kolbeck R, Sanjuan MA (2016) Self-reactive IgE exacerbates interferon responses associated with autoimmunity. *Nat Immunol* 17: 196-203

Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G (2007) HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 48: 124-31

Herrinton LJ, Liu L, Goldfien R, Michaels MA, Tran TN (2016) Risk of Serious Infection for Patients with Systemic Lupus Erythematosus Starting Glucocorticoids with or without Antimalarials. *J Rheumatol* 43: 1503-9

Hochberg MC (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 40: 1725

Holmqvist M, Simard JF, Asplund K, Arkema EV (2015) Stroke in systemic lupus erythematosus: a meta-analysis of population-based cohort studies. *RMD Open* 1: e000168

Hsiao YP, Tsai JD, Muo CH, Tsai CH, Sung FC, Liao YT, Chang YJ, Yang JH (2014) Atopic diseases and systemic lupus erythematosus: an epidemiological study of the risks and correlations. *Int J Environ Res Public Health* 11: 8112-22

Hsu CY, Lin YS, Su YJ, Lin HF, Lin MS, Syu YJ, Cheng TT, Yu SF, Chen JF, Chen TH (2017) Effect of long-term hydroxychloroquine on vascular events in patients with systemic lupus erythematosus: a database prospective cohort study. *Rheumatology (Oxford)* 56: 2212-2221

Hu N, Qiu X, Luo Y, Yuan J, Li Y, Lei W, Zhang G, Zhou Y, Su Y, Lu Q (2008) Abnormal histone modification patterns in lupus CD4+ T cells. *J Rheumatol* 35: 804-10

Hughes T, Adler A, Merrill JT, Kelly JA, Kaufman KM, Williams A, Langefeld CD, Gilkeson GS, Sanchez E, Martin J, Boackle SA, Stevens AM, Alarcon GS, Niewold TB, Brown EE, Kimberly RP, Edberg JC, Ramsey-Goldman R, Petri M, Reveille JD et al. (2012) Analysis of autosomal genes reveals gene-sex interactions and higher total genetic risk in men with systemic lupus erythematosus. *Ann Rheum Dis* 71: 694-9

Huscher D, Merkesdal S, Thiele K, Zeidler H, Schneider M, Zink A, German Collaborative Arthritis C (2006) Cost of illness in rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis and systemic lupus erythematosus in Germany. *Ann Rheum Dis* 65: 1175-83

Iaccarino L, Bettio S, Reggia R, Zen M, Frassi M, Andreoli L, Gatto M, Piantoni S, Nalotto L, Franceschini F, Larosa M, Fredi M, Punzi L, Tincani A, Doria A (2017) Effects of Belimumab on Flare Rate and Expected Damage Progression in Patients With Active Systemic Lupus Erythematosus. *Arthritis Care Res (Hoboken)* 69: 115-123

Ibanez D, Urowitz MB, Gladman DD (2003) Summarizing disease features over time: I. Adjusted mean SLEDAI derivation and application to an index of disease activity in lupus. *J Rheumatol* 30: 1977-82

International Union of Immunological Societies Expert Committee on Primary I, Notarangelo LD, Fischer A, Geha RS, Casanova JL, Chapel H, Conley ME, Cunningham-Rundles C, Etzioni A, Hammartrom L, Nonoyama S, Ochs HD, Puck J, Roifman C, Seger

R, Wedgwood J (2009) Primary immunodeficiencies: 2009 update. *J Allergy Clin Immunol* 124: 1161-78

Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A (2007) Fifty years of anti-ds DNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 46: 1052-6

Isenberg DA, Rahman A, Allen E, Farewell V, Akil M, Bruce IN, D'Cruz D, Griffiths B, Khamashta M, Maddison P, McHugh N, Snaith M, Teh LS, Yee CS, Zoma A, Gordon C (2005) BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 44: 902-6

Ivashkiv LB (2013) PTPN22 in autoimmunity: different cell and different way. *Immunity* 39: 91-3

Jacobs BM, Giovannoni G, Cuzick J, Dobson R (2020) Systematic review and meta-analysis of the association between Epstein-Barr virus, multiple sclerosis and other risk factors. *Mult Scler* 26: 1281-1297

James EA, Rieck M, Pieper J, Gebe JA, Yue BB, Tatum M, Peda M, Sandin C, Klareskog L, Malmstrom V, Buckner JH (2014) Citrulline-specific Th1 cells are increased in rheumatoid arthritis and their frequency is influenced by disease duration and therapy. *Arthritis & rheumatology* 66: 1712-22

James JA, Kaufman KM, Farris AD, Taylor-Albert E, Lehman TJ, Harley JB (1997) An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. *J Clin Invest* 100: 3019-26

Jasemi S, Erre GL, Cadoni ML, Bo M, Sechi LA (2021) Humoral Response to Microbial Biomarkers in Rheumatoid Arthritis Patients. *J Clin Med* 10

Jego G, Palucka AK, Blanck JP, Chalouni C, Pascual V, Banchereau J (2003) Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* 19: 225-34

Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, Flores-Suarez LF, Gross WL, Guillevin L, Hagen EC, Hoffman GS, Jayne DR, Kallenberg CG, Lamprecht P, Langford CA, Luqmani RA, Mahr AD, Matteson EL, Merkel PA, Ozen S et al. (2013) 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 65: 1-11

Jeong HW, Her M, Bae JS, Kim SK, Lee SW, Kim HK, Kim D, Park N, Chung WT, Lee SY, Choe JY, Kim IJ (2015) Brain MRI in neuropsychiatric lupus: associations with the 1999 ACR case definitions. *Rheumatol Int* 35: 861-9

Jesus D, Matos A, Henriques C, Zen M, Larosa M, Iaccarino L, Da Silva JAP, Doria A, Ines LS (2019) Derivation and validation of the SLE Disease Activity Score (SLE-DAS): a new SLE continuous measure with high sensitivity for changes in disease activity. *Ann Rheum Dis* 78: 365-371

Jin X, Han Y, Wang JQ, Lu L (2021) CAR-T cell therapy: new hope for systemic lupus erythematosus patients. *Cell Mol Immunol* 18: 2581-2582

Joo YB, Kim KJ, Park KS, Park YJ (2021) Influenza infection as a trigger for systemic lupus erythematosus flares resulting in hospitalization. *Sci Rep* 11: 1-7

Joseph A, Prasad S, Hynan LS, Chren MM, Chong B (2021) Differences in quality of life in patients with cutaneous lupus erythematosus with varying income levels. *Lupus* 30: 972-980

Joseph JE, Harrison P, Mackie IJ, Isenberg DA, Machin SJ (2001) Increased circulating platelet-leucocyte complexes and platelet activation in patients with antiphospholipid syndrome, systemic lupus erythematosus and rheumatoid arthritis. *Br J Haematol* 115: 451-9

Kachuri L, Francis SS, Morrison ML, Wendt GA, Bosse Y, Cavazos TB, Rashkin SR, Ziv E, Witte JS (2020) The landscape of host genetic factors involved in immune response to common viral infections. *Genome Med* 12: 1-18

Kaczmarczyk-Sekula K, Dyduch G, Kostanski M, Wielowieyska-Szybinska D, Szpor J, Bialas M, Okon K (2015) Mast cells in systemic and cutaneous lupus erythematosus. *Pol J Pathol* 66: 397-402

Kain R, Exner M, Brandes R, Ziebermayr R, Cunningham D, Alderson CA, Davidovits A, Raab I, Jahn R, Ashour O, Spitzauer S, Sunder-Plassmann G, Fukuda M, Klemm P, Rees AJ, Kerjaschki D (2008) Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat Med* 14: 1088-96

Kalekar LA, Schmiel SE, Nandiwada SL, Lam WY, Barsness LO, Zhang N, Stritesky GL, Malhotra D, Pauken KE, Linehan JL, O'Sullivan MG, Fife BT, Hogquist KA, Jenkins MK, Mueller DL (2016) CD4(+) T cell anergy prevents autoimmunity and generates regulatory T cell precursors. *Nat Immunol* 17: 304-14

Kallenberg CG (2011) Pathogenesis of ANCA-associated vasculitides. *Ann Rheum Dis* 70 Suppl 1: i59-63

Kalluri SR, Grummel V, Hracsko Z, Pongratz V, Pernpeintner V, Gasperi C, Buck D, Hemmer B, Consortium A (2018) Interferon-beta specific T cells are associated with the development of neutralizing antibodies in interferon-beta treated multiple sclerosis patients. *J Autoimmun* 88: 83-90

Kang I, Quan T, Nolasco H, Park SH, Hong MS, Crouch J, Pamer EG, Howe JG, Craft J (2004) Defective control of latent Epstein-Barr virus infection in systemic lupus erythematosus. *J Immunol* 172: 1287-94

Katsuyama T, Tsokos GC, Moulton VR (2018) Aberrant T Cell Signaling and Subsets in Systemic Lupus Erythematosus. *Front Immunol* 9: 1088

Kattah NH, Newell EW, Jarrell JA, Chu AD, Xie J, Kattah MG, Goldberger O, Ye J, Chakravarty EF, Davis MM, Utz PJ (2015) Tetramers reveal IL-17-secreting CD4+ T cells that are specific for U1-70 in lupus and mixed connective tissue disease. *Proc Natl Acad Sci U S A* 112: 3044-9

Kawase T, Tanaka H, Kojima H, Uchida N, Ohashi K, Fukuda T, Ozawa Y, Ikegame K, Eto T, Mori T, Miyamoto T, Hidaka M, Shiratori S, Takanashi M, Atsuta Y, Ichinohe T, Kanda Y, Kanda J, Transplantation HLAWGotJSfHC (2019) Impact of High-Frequency HLA Haplotypes on Clinical Cytomegalovirus Reactivation in Allogeneic Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant* 25: 2482-2489

Khadjinova AI, Wang X, Laine A, Ukadike K, Eckert M, Stevens A, Bengtsson AA, Lood C, Mustelin T (2021) Autoantibodies against the envelope proteins of endogenous retroviruses K102 and K108 in patients with systemic lupus erythematosus correlate with active disease. *Clin Exp Rheumatol*

Khamashta M, Merrill JT, Werth VP, Furie R, Kalunian K, Illei GG, Drappa J, Wang L, Greth W, investigators CDs (2016) Sifalimumab, an anti-interferon-alpha monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. *Ann Rheum Dis* 75: 1909-1916

Kidney Disease: Improving Global Outcomes Glomerular Diseases Work G (2021) KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. *Kidney Int* 100: S1-S276

Kim-Howard X, Maiti AK, Anaya JM, Bruner GR, Brown E, Merrill JT, Edberg JC, Petri MA, Reveille JD, Ramsey-Goldman R, Alarcon GS, Vyse TJ, Gilkeson G, Kimberly RP, James JA, Guthridge JM, Harley JB, Nath SK (2010) ITGAM coding variant (rs1143679) influences the risk of renal disease, discoid rash and immunological manifestations in patients with systemic lupus erythematosus with European ancestry. *Ann Rheum Dis* 69: 1329-32

Kim JM, Kwok SK, Ju JH, Kim HY, Park SH (2012) Reactive hemophagocytic syndrome in adult Korean patients with systemic lupus erythematosus: a case-control study and literature review. *J Rheumatol* 39: 86-93

Klein RS, Morganroth PA, Werth VP (2010) Cutaneous lupus and the Cutaneous Lupus Erythematosus Disease Area and Severity Index instrument. *Rheum Dis Clin North Am* 36: 33-51, vii

Kolkhir P, Pogorelov D, Olisova O, Maurer M (2016) Comorbidity and pathogenic links of chronic spontaneous urticaria and systemic lupus erythematosus--a systematic review. *Clin Exp Allergy* 46: 275-87

Kronzer VL, Crowson CS, Sparks JA, Vassallo R, Davis JM, 3rd (2019) Investigating Asthma, Allergic Disease, Passive Smoke Exposure, and Risk of Rheumatoid Arthritis. *Arthritis & rheumatology* 71: 1217-1224

Krouwels FH, Hol BE, Lutter R, Bruinier B, Bast A, Jansen HM, Out TA (1998) Histamine affects interleukin-4, interleukin-5, and interferon-gamma production by human T cell clones from the airways and blood. *Am J Respir Cell Mol Biol* 18: 721-30

Kubo S, Nakayamada S, Yoshikawa M, Miyazaki Y, Sakata K, Nakano K, Hanami K, Iwata S, Miyagawa I, Saito K, Tanaka Y (2017) Peripheral Immunophenotyping Identifies Three Subgroups Based on T Cell Heterogeneity in Lupus Patients. *Arthritis & rheumatology* 69: 2029-2037

Kuhn A, Landmann A (2014) The classification and diagnosis of cutaneous lupus erythematosus. *J Autoimmun* 48-49: 14-9

Kwok WW, Roti M, Delong JH, Tan V, Wambre E, James EA, Robinson D (2010) Direct ex vivo analysis of allergen-specific CD4+ T cells. *J Allergy Clin Immunol* 125: 1407-1409 e1

Langen U, Schmitz R, Steppuhn H (2013) [Prevalence of allergic diseases in Germany: results of the German Health Interview and Examination Survey for Adults (DEGS1)]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 56: 698-706

Lara-Marquez ML, Yunis JJ, Layrisse Z, Ortega F, Carvallo-Gil E, Montagnani S, Makhatadze NJ, Pocino M, Granja C, Yunis E (1999) Immunogenetics of atopic asthma: association of DRB1*1101 DQA1*0501 DQB1*0301 haplotype with Dermatophagoides spp.-sensitive asthma in a sample of the Venezuelan population. *Clin Exp Allergy* 29: 60-71

Lee HY, Hong YK, Yun HJ, Kim YM, Kim JR, Yoo WH (2008) Altered frequency and migration capacity of CD4+CD25+ regulatory T cells in systemic lupus erythematosus. *Rheumatology (Oxford)* 47: 789-94

Lee YH, Lee HS, Choi SJ, Ji JD, Song GG (2012) Associations between TLR polymorphisms and systemic lupus erythematosus: a systematic review and meta-analysis. *Clin Exp Rheumatol* 30: 262-5

Lee YJ, Park JA, Kwon H, Choi YS, Jung KC, Park SH, Lee EB (2018) Role of Stem Cell-Like Memory T Cells in Systemic Lupus Erythematosus. *Arthritis & rheumatology* 70: 1459-1469

Leffler J, Martin M, Gullstrand B, Tyden H, Lood C, Truedsson L, Bengtsson AA, Blom AM (2012) Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. *J Immunol* 188: 3522-31

Leogrande G, Jirillo E (1993) Studies on the epidemiology of child infections in the Bari area (south Italy). VII. Epidemiology of Epstein-Barr virus infections. *Eur J Epidemiol* 9: 368-72

Leu SJ, Lee TY, Cheng SW, Tsai MY, Lin YS, Chiou TJ, Huang KY, Chiang AN (2019) Structural and functional characterization of beta2 -glycoprotein I domain 1 in anti-melanoma cell migration. *Cancer Sci* 110: 1974-1986

Lever E, Alves MR, Isenberg DA (2020) Towards Precision Medicine in Systemic Lupus Erythematosus. *Pharmgenomics Pers Med* 13: 39-49

Li PH, Yeung HHF, Lau CS, Au EYL (2020) Prevalence, Incidence, and Sensitization Profile of beta-lactam Antibiotic Allergy in Hong Kong. *JAMA Netw Open* 3: e204199

Li Y, Harada T, Juang YT, Kyttaris VC, Wang Y, Zidanic M, Tung K, Tsokos GC (2007) Phosphorylated ERM is responsible for increased T cell polarization, adhesion, and migration in patients with systemic lupus erythematosus. *J Immunol* 178: 1938-47

Li Z, Xu D, Wang Z, Wang Y, Zhang S, Li M, Zeng X (2017) Gastrointestinal system involvement in systemic lupus erythematosus. *Lupus* 26: 1127-1138

Liblau RS, Gonzalez-Dunia D, Wiendl H, Zipp F (2013) Neurons as targets for T cells in the nervous system. *Trends Neurosci* 36: 315-24

Lim LSH, Pullenayegum E, Feldman BM, Lim L, Gladman DD, Silverman ED (2018) From Childhood to Adulthood: Disease Activity Trajectories in Childhood-Onset Systemic Lupus Erythematosus. *Arthritis Care Res (Hoboken)* 70: 750-757

Liszewski MK, Atkinson JP (2011) Too much of a good thing at the site of tissue injury: the instructive example of the complement system predisposing to thrombotic microangiopathy. *Hematology Am Soc Hematol Educ Program* 2011: 9-14

Liu J, Harberts E, Tammaro A, Girardi N, Filler RB, Fishelevich R, Temann A, Licona-Limon P, Girardi M, Flavell RA, Gaspari AA (2014) IL-9 regulates allergen-specific Th1 responses in allergic contact dermatitis. *J Invest Dermatol* 134: 1903-1911

Lloyd RE, Tamhankar M, Lernmark A (2021) Enteroviruses and Type 1 Diabetes: Multiple Mechanisms and Factors? *Annu Rev Med*

Long HM, Chagoury OL, Leese AM, Ryan GB, James E, Morton LT, Abbott RJ, Sabbah S, Kwok W, Rickinson AB (2013) MHC II tetramers visualize human CD4+ T cell responses to Epstein-Barr virus infection and demonstrate atypical kinetics of the nuclear antigen EBNA1 response. *J Exp Med* 210: 933-49

Lood C, Blanco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, Smith CK, Malech HL, Ledbetter JA, Elkon KB, Kaplan MJ (2016) Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat Med* 22: 146-53

Louahed J, Zhou Y, Maloy WL, Rani PU, Weiss C, Tomer Y, Vink A, Renauld J, Van Snick J, Nicolaides NC, Levitt RC, Haczku A (2001) Interleukin 9 promotes influx and local maturation of eosinophils. *Blood* 97: 1035-42

Lu L, Kaliyaperumal A, Boumpas DT, Datta SK (1999) Major peptide autoepitopes for nucleosome-specific T cells of human lupus. *J Clin Invest* 104: 345-55

Lu Y, Yoo BK, Ng AHC, Kim J, Yeom S, Tang J, Lin MM, Zewail AH, Heath JR (2019) 4D electron microscopy of T cell activation. *Proc Natl Acad Sci U S A* 116: 22014-22019

Luijten KM, Tekstra J, Bijlsma JW, Bijl M (2012) The Systemic Lupus Erythematosus Responder Index (SRI); a new SLE disease activity assessment. *Autoimmunity reviews* 11: 326-9

Lundstrom E, Gustafsson JT, Jonsen A, Leonard D, Zickert A, Elvin K, Sturfelt G, Nordmark G, Bengtsson AA, Sundin U, Kallberg H, Sandling JK, Syvanen AC, Klareskog L, Gunnarsson I, Ronnblom L, Padyukov L, Svenungsson E (2013) HLA-DRB1*04/*13 alleles are associated with vascular disease and antiphospholipid antibodies in systemic lupus erythematosus. *Ann Rheum Dis* 72: 1018-25

Luo Y, Fan X, Jiang C, Ramos-Rodriguez A, Wen Y, Zhang J, Huang F, Guan X, Xu J (2019) Systemic Lupus Erythematosus and Angioedema: A Cross-Sectional Study From the National Inpatient Sample. *Arch Rheumatol* 34: 301-307

Lv N, Wang Z, Dang A, Zhu X, Liu Y, Zheng D, Liu G (2015) HLA-DQA1, DQB1 and DRB1 alleles associated with Takayasu arteritis in the Chinese Han population. *Hum Immunol* 76: 241-4

Macaubas C, Wahlstrom J, Galvao da Silva AP, Forsthuber TG, Sonderstrup G, Kwok WW, DeKruyff RH, Umetsu DT (2006) Allergen-specific MHC class II tetramer+ cells are detectable in allergic, but not in nonallergic, individuals. *J Immunol* 176: 5069-77

Maiti AK, Kim-Howard X, Motghare P, Pradhan V, Chua KH, Sun C, Arango-Guerrero MT, Ghosh K, Niewold TB, Harley JB, Anaya JM, Looger LL, Nath SK (2014) Combined protein- and nucleic acid-level effects of rs1143679 (R77H), a lupus-predisposing variant within ITGAM. *Hum Mol Genet* 23: 4161-76

Maldonado A, Mueller YM, Thomas P, Bojczuk P, O'Connors C, Katsikis PD (2003) Decreased effector memory CD45RA+ CD62L- CD8+ T cells and increased central memory CD45RA- CD62L+ CD8+ T cells in peripheral blood of rheumatoid arthritis patients. *Arthritis Res Ther* 5: R91-6

Mammen AL, Gaudet D, Brisson D, Christopher-Stine L, Lloyd TE, Leffell MS, Zachary AA (2012) Increased frequency of DRB1*11:01 in anti-hydroxymethylglutaryl-coenzyme A reductase-associated autoimmune myopathy. *Arthritis Care Res (Hoboken)* 64: 1233-7

Manfredi AA, Covino C, Rovere-Querini P, Maugeri N (2015) Instructive influences of phagocytic clearance of dying cells on neutrophil extracellular trap generation. *Clin Exp Immunol* 179: 24-9

Manfredi AA, Ramirez GA, Rovere-Querini P, Maugeri N (2018) The Neutrophil's Choice: Phagocytose vs Make Neutrophil Extracellular Traps. *Front Immunol* 9: 288

Manfredi AA, Rovere-Querini P, Maugeri N (2010) Dangerous connections: neutrophils and the phagocytic clearance of activated platelets. *Curr Opin Hematol* 17: 3-8

Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* 11: 519-31

Manzi S, Sanchez-Guerrero J, Merrill JT, Furie R, Gladman D, Navarra SV, Ginzler EM, D'Cruz DP, Doria A, Cooper S, Zhong ZJ, Hough D, Freimuth W, Petri MA, Bliss, Groups B-S (2012) Effects of belimumab, a B lymphocyte stimulator-specific inhibitor, on disease activity across multiple organ domains in patients with systemic lupus erythematosus: combined results from two phase III trials. *Ann Rheum Dis* 71: 1833-8

Marchesini G, Marchignoli F, Petta S (2017) Evidence-Based Medicine and the Problem of Healthy Volunteers. *Ann Hepatol* 16: 832-834

Maruyama A, Nagashima T, Iwamoto M, Minota S (2018) Clinical characteristics of lupus enteritis in Japanese patients: the large intestine-dominant type has features of intestinal pseudo-obstruction. *Lupus* 27: 1661-1669

Massa M, Mazzoli F, Pignatti P, De Benedetti F, Passalia M, Viola S, Samodal R, La Cava A, Giannoni F, Ollier W, Martini A, Albani S (2002) Proinflammatory responses to self HLA epitopes are triggered by molecular mimicry to Epstein-Barr virus proteins in oligoarticular juvenile idiopathic arthritis. *Arthritis Rheum* 46: 2721-9

Massilamany C, Upadhyaya B, Gangaplara A, Kuszynski C, Reddy J (2011) Detection of autoreactive CD4 T cells using major histocompatibility complex class II dextramers. *BMC Immunol* 12: 40

Maugeri N, Campana L, Gavina M, Covino C, De Metrio M, Panciroli C, Maiuri L, Maseri A, D'Angelo A, Bianchi ME (2014) Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. *J Thromb Haemost* 12: 2074-2088

Maugeri N, Cattaneo M, Rovere-Querini P, Manfredi AA (2014) Platelet clearance by circulating leukocytes: a rare event or a determinant of the "immune continuum"? *Platelets* 25: 224-5

McCarthy EM, Moreno-Martinez D, Wilkinson FL, McHugh NJ, Bruce IN, Pauling JD, Alexander MY, Parker B (2017) Microparticle subpopulations are potential markers of disease progression and vascular dysfunction across a spectrum of connective tissue disease. *BBA Clin* 7: 16-22

McKenna K, Beignon AS, Bhardwaj N (2005) Plasmacytoid dendritic cells: linking innate and adaptive immunity. *J Virol* 79: 17-27

McWilliam V, Koplin J, Lodge C, Tang M, Dharmage S, Allen K (2015) The Prevalence of Tree Nut Allergy: A Systematic Review. *Curr Allergy Asthma Rep* 15: 54

Meckiff BJ, Ladell K, McLaren JE, Ryan GB, Leese AM, James EA, Price DA, Long HM (2019) Primary EBV Infection Induces an Acute Wave of Activated Antigen-Specific Cytotoxic CD4(+) T Cells. *J Immunol* 203: 1276-1287

Menon M, Blair PA, Isenberg DA, Mauri C (2016) A regulatory feedback between plasmacytoid dendritic cells and regulatory B cells is aberrant in systemic lupus erythematosus. *Immunity* 44: 683-697

Merrill JT, Neuwelt CM, Wallace DJ, Shanahan JC, Latinis KM, Oates JC, Utset TO, Gordon C, Isenberg DA, Hsieh HJ, Zhang D, Brunetta PG (2010) Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum* 62: 222-33

Milone MC, Xu J, Chen SJ, Collins MA, Zhou J, Powell DJ, Jr., Melenhorst JJ (2021) Engineering enhanced CAR T-cells for improved cancer therapy. *Nat Cancer* 2: 780-793

Mirakian R, Leech SC, Krishna MT, Richter AG, Huber PA, Farooque S, Khan N, Pirmohamed M, Clark AT, Nasser SM, Standards of Care Committee of the British Society for A, Clinical I (2015) Management of allergy to penicillins and other beta-lactams. *Clin Exp Allergy* 45: 300-27

Misra R, Danda D, Rajappa SM, Ghosh A, Gupta R, Mahendranath KM, Jeyaseelan L, Lawrence A, Bacon PA, group IRV (2013) Development and initial validation of the Indian Takayasu Clinical Activity Score (ITAS2010). *Rheumatology (Oxford)* 52: 1795-801

Miyagawa S, Shinohara K, Kidoguchi K, Fujita T, Fukumoto T, Hashimoto K, Yoshioka A, Shirai T (1997) Neonatal lupus erythematosus: studies on HLA class II genes and autoantibody profiles in Japanese mothers. *Autoimmunity* 26: 95-101

Miyake K, Akahoshi M, Nakashima H (2011) Th subset balance in lupus nephritis. *J Biomed Biotechnol* 2011: 980286

Mohan C, Putterman C (2015) Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. *Nat Rev Nephrol* 11: 329-41

Monneaux F, Briand JP, Muller S (2000) B and T cell immune response to small nuclear ribonucleoprotein particles in lupus mice: autoreactive CD4(+) T cells recognize a T cell epitope located within the RNP80 motif of the 70K protein. *Eur J Immunol* 30: 2191-200

Monneaux F, Hoebeke J, Sordet C, Nonn C, Briand JP, Maillere B, Sibillia J, Muller S (2005) Selective modulation of CD4+ T cells from lupus patients by a promiscuous, protective peptide analog. *J Immunol* 175: 5839-47

Monneaux F, Muller S (2002) Epitope spreading in systemic lupus erythematosus: identification of triggering peptide sequences. *Arthritis Rheum* 46: 1430-8

Moon UY, Park SJ, Oh ST, Kim WU, Park SH, Lee SH, Cho CS, Kim HY, Lee WK, Lee SK (2004) Patients with systemic lupus erythematosus have abnormally elevated Epstein-Barr virus load in blood. *Arthritis Res Ther* 6: R295-302

Morand EF, Furie R, Tanaka Y, Bruce IN, Askanase AD, Richez C, Bae SC, Brohawn PZ, Pineda L, Berglind A, Tummala R, Investigators T-T (2020) Trial of Anifrolumab in Active Systemic Lupus Erythematosus. *N Engl J Med* 382: 211-221

Morikawa H, Ohkura N, Vandenbon A, Itoh M, Nagao-Sato S, Kawaji H, Lassmann T, Carninci P, Hayashizaki Y, Forrest AR, Standley DM, Date H, Sakaguchi S, Consortium F (2014) Differential roles of epigenetic changes and Foxp3 expression in regulatory T cell-specific transcriptional regulation. *Proc Natl Acad Sci U S A* 111: 5289-94

Moroni G, Gallelli B, Quaglini S, Banfi G, Rivolta E, Messa P, Ponticelli C (2006) Withdrawal of therapy in patients with proliferative lupus nephritis: long-term follow-up. *Nephrol Dial Transplant* 21: 1541-8

Moroni L, Mazzetti M, Ramirez GA, Farina N, Bozzolo EP, Guerrieri S, Moiola L, Filippi M, Di Mattei V, Dagna L (2021) Beyond Neuropsychiatric Manifestations of

Systemic Lupus Erythematosus: Focus on Post-traumatic Stress Disorder and Alexithymia. *Curr Rheumatol Rep* 23: 52

Morris DL, Fernando MM, Taylor KE, Chung SA, Nititham J, Alarcon-Riquelme ME, Barcellos LF, Behrens TW, Cotsapas C, Gaffney PM, Graham RR, Pons-Estel BA, Gregersen PK, Harley JB, Hauser SL, Hom G, Langefeld CD, Noble JA, Rioux JD, Seldin MF et al. (2014) MHC associations with clinical and autoantibody manifestations in European SLE. *Genes Immun* 15: 210-7

Morris DL, Taylor KE, Fernando MM, Nititham J, Alarcon-Riquelme ME, Barcellos LF, Behrens TW, Cotsapas C, Gaffney PM, Graham RR, Pons-Estel BA, Gregersen PK, Harley JB, Hauser SL, Hom G, International MHC, Autoimmunity Genetics N, Langefeld CD, Noble JA, Rioux JD et al. (2012) Unraveling multiple MHC gene associations with systemic lupus erythematosus: model choice indicates a role for HLA alleles and non-HLA genes in Europeans. *Am J Hum Genet* 91: 778-93

Morton S, Palmer B, Muir K, Powell RJ (1998) IgE and non-IgE mediated allergic disorders in systemic lupus erythematosus. *Ann Rheum Dis* 57: 660-3

Mosca M, Bencivelli W, Vitali C, Carrai P, Neri R, Bombardieri S (2000) The validity of the ECLAM index for the retrospective evaluation of disease activity in systemic lupus erythematosus. *Lupus* 9: 445-50

Mougiakakos D, Kronke G, Volkl S, Kretschmann S, Aigner M, Kharboutli S, Boltz S, Manger B, Mackensen A, Schett G (2021) CD19-Targeted CAR T Cells in Refractory Systemic Lupus Erythematosus. *N Engl J Med* 385: 567-569

Munz C, Bickham KL, Subklewe M, Tsang ML, Chahroudi A, Kurilla MG, Zhang D, O'Donnell M, Steinman RM (2000) Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. *J Exp Med* 191: 1649-60

Muro M, Mondejar-Lopez P, Moya-Quiles MR, Salgado G, Pastor-Vivero MD, Lopez-Hernandez R, Boix F, Campillo JA, Minguela A, Garcia-Alonso A, Sanchez-Solis M, Alvarez-Lopez MR (2013) HLA-DRB1 and HLA-DQB1 genes on susceptibility to and protection from allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Microbiol Immunol* 57: 193-7

Naji N, Smith SG, Gauvreau GM, O'Byrne PM (2014) T helper 17 cells and related cytokines after allergen inhalation challenge in allergic asthmatics. *Int Arch Allergy Immunol* 165: 27-34

Nakagome K, Nagata M (2021) Allergen Immunotherapy in Asthma. *Pathogens* 10

Navarro-Zarza JE, Alvarez-Hernandez E, Casasola-Vargas JC, Estrada-Castro E, Burgos-Vargas R (2010) Prevalence of community-acquired and nosocomial infections in hospitalized patients with systemic lupus erythematosus. *Lupus* 19: 43-8

Nhim C, Delluc S, Halgand F, de Chaisemartin L, Weaver RJ, Claude N, Joseph D, Maillere B, Pallardy M (2013) Identification and frequency of circulating CD4(+) T lymphocytes specific to Benzylpenicillin in healthy donors. *Allergy* 68: 899-905

Niewold TB, Kelly JA, Flesch MH, Espinoza LR, Harley JB, Crow MK (2008) Association of the IRF5 risk haplotype with high serum interferon-alpha activity in systemic lupus erythematosus patients. *Arthritis Rheum* 58: 2481-7

Niewold TB, Kelly JA, Kariuki SN, Franek BS, Kumar AA, Kaufman KM, Thomas K, Walker D, Kamp S, Frost JM, Wong AK, Merrill JT, Alarcon-Riquelme ME, Tikly M, Ramsey-Goldman R, Reveille JD, Petri MA, Edberg JC, Kimberly RP, Alarcon GS et al. (2012) IRF5 haplotypes demonstrate diverse serological associations which predict serum interferon alpha activity and explain the majority of the genetic association with systemic lupus erythematosus. *Ann Rheum Dis* 71: 463-8

Nittner-Marszalska M, Rosiek-Biegus M, Kopec A, Pawlowicz R, Kosinska M, Lata A, Szenborn L (2021) Pfizer-BioNTech COVID-19 Vaccine Tolerance in Allergic versus Non-Allergic Individuals. *Vaccines (Basel)* 9

Niu Z, Zhang P, Tong Y (2015) Value of HLA-DR genotype in systemic lupus erythematosus and lupus nephritis: a meta-analysis. *Int J Rheum Dis* 18: 17-28

Nossent J, Cikes N, Kiss E, Marchesoni A, Nassonova V, Mosca M, Olesinska M, Pokorny G, Rozman B, Schneider M, Vlachoyiannopoulos PG, Swaak A (2007) Current causes of death in systemic lupus erythematosus in Europe, 2000--2004: relation to disease activity and damage accrual. *Lupus* 16: 309-17

Nossent JC (1998) SLICC/ACR Damage Index in Afro-Caribbean patients with systemic lupus erythematosus: changes in and relationship to disease activity, corticosteroid therapy, and prognosis. *J Rheumatol* 25: 654-9

Novelli A, Andreani M, Biancolella M, Liberatoscioli L, Passarelli C, Colona VL, Rogliani P, Leonardis F, Campana A, Carsetti R, Andreoni M, Bernardini S, Novelli G, Locatelli F (2020) HLA allele frequencies and susceptibility to COVID-19 in a group of 99 Italian patients. *HLA* 96: 610-614

O'Hanlon TP, Carrick DM, Targoff IN, Arnett FC, Reveille JD, Carrington M, Gao X, Oddis CV, Morel PA, Malley JD, Malley K, Shamim EA, Rider LG, Chanock SJ, Foster CB, Bunch T, Blackshear PJ, Plotz PH, Love LA, Miller FW (2006) Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A, -B, -Cw, -DRB1, and -DQA1 allelic profiles distinguish European American patients with different myositis autoantibodies. *Medicine (Baltimore)* 85: 111-127

Oglesby A, Korves C, Laliberte F, Dennis G, Rao S, Suthoff ED, Wei R, Duh MS (2014) Impact of early versus late systemic lupus erythematosus diagnosis on clinical and economic outcomes. *Appl Health Econ Health Policy* 12: 179-90

Oh BR, Chen P, Nidetz R, McHugh W, Fu J, Shanley TP, Cornell TT, Kurabayashi K (2016) Multiplexed Nanoplasmonic Temporal Profiling of T-Cell Response under Immunomodulatory Agent Exposure. *ACS Sens* 1: 941-948

Ohshima Y, Yang LP, Avice MN, Kurimoto M, Nakajima T, Sergerie M, Demeure CE, Sarfati M, Delespesse G (1999) Naive human CD4+ T cells are a major source of lymphotoxin alpha. *J Immunol* 162: 3790-4

Oliveira G, Ruggiero E, Stanghellini MT, Cieri N, D'Agostino M, Fronza R, Lulay C, Dionisio F, Mastaglio S, Greco R, Peccatori J, Aiuti A, Ambrosi A, Biasco L, Bondanza A, Lambiase A, Traversari C, Vago L, von Kalle C, Schmidt M et al. (2015) Tracking genetically engineered lymphocytes long-term reveals the dynamics of T cell immunological memory. *Sci Transl Med* 7: 317ra198

Olivieri M, Verlato G, Corsico A, Lo Cascio V, Bugiani M, Marinoni A, de Marco R, Italian European Community Respiratory Health Survey g (2002) Prevalence and features of allergic rhinitis in Italy. *Allergy* 57: 600-6

Ostergaard O, Nielsen CT, Tanassi JT, Iversen LV, Jacobsen S, Heegaard NHH (2017) Distinct proteome pathology of circulating microparticles in systemic lupus erythematosus. *Clin Proteomics* 14: 23

Pablos JL, Abasolo L, Alvaro-Gracia JM, Blanco FJ, Blanco R, Castrejon I, Fernandez-Fernandez D, Fernandez-Gutierrez B, Galindo-Izquierdo M, Gonzalez-Gay MA, Manrique-Arija S, Mena Vazquez N, Mera Varela A, Retuerto M, Seijas-Lopez A, group Ri (2020) Prevalence of hospital PCR-confirmed COVID-19 cases in patients with chronic inflammatory and autoimmune rheumatic diseases. *Ann Rheum Dis* 79: 1170-1173

Padovan E, Bauer T, Tongio MM, Kalbacher H, Weltzien HU (1997) Penicilloyl peptides are recognized as T cell antigenic determinants in penicillin allergy. *Eur J Immunol* 27: 1303-7

Pallikkuth S, Williams E, Pahwa R, Hoffer M, Pahwa S (2021) Association of Flu specific and SARS-CoV-2 specific CD4 T cell responses in SARS-CoV-2 infected asymptomatic heath care workers. *Vaccine* 39: 6019-6024

Pan Q, Feng Y, Peng Y, Zhou H, Deng Z, Li L, Han H, Lin J, Shi L, Wang S, An N, Yang C, Liu HF (2017) Basophil Recruitment to Skin Lesions of Patients with Systemic Lupus Erythematosus Mediated by CCR1 and CCR2. *Cell Physiol Biochem* 43: 832-839

Pang M, Setoyama Y, Tsuzaka K, Yoshimoto K, Amano K, Abe T, Takeuchi T (2002) Defective expression and tyrosine phosphorylation of the T cell receptor zeta chain in peripheral blood T cells from systemic lupus erythematosus patients. *Clin Exp Immunol* 129: 160-8

Pardi N, Hogan MJ, Porter FW, Weissman D (2018) mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov* 17: 261-279

Park H, Ahn K, Park MH, Lee SI (2012) The HLA-DRB1 Polymorphism is Associated With Atopic Dermatitis, but not Egg Allergy in Korean Children. *Allergy Asthma Immunol Res* 4: 143-9

Parks CG, Biagini RE, Cooper GS, Gilkeson GS, Dooley MA (2010) Total serum IgE levels in systemic lupus erythematosus and associations with childhood onset allergies. *Lupus* 19: 1614-22

Parlesak A, Haller D, Brinz S, Baeuerlein A, Bode C (2004) Modulation of cytokine release by differentiated CACO-2 cells in a compartmentalized coculture model with mononuclear leucocytes and nonpathogenic bacteria. *Scand J Immunol* 60: 477-85

Pastorello EA, Rivolta F, Bianchi M, Mauro M, Pravettoni V (2001) Incidence of anaphylaxis in the emergency department of a general hospital in Milan. *J Chromatogr B Biomed Sci Appl* 756: 11-7

Paul S, Lindestam Arlehamn CS, Scriba TJ, Dillon MB, Oseroff C, Hinz D, McKinney DM, Carrasco Pro S, Sidney J, Peters B, Sette A (2015) Development and validation of a broad scheme for prediction of HLA class II restricted T cell epitopes. *J Immunol Methods* 422: 28-34

Pawankar R, Canonica G, Holgate S, Lockey R, Blaiss MJMWAO (2013) WAO white book on allergy: update 2013.

Pego-Reigosa JM, Medeiros DA, Isenberg DA (2009) Respiratory manifestations of systemic lupus erythematosus: old and new concepts. *Best Pract Res Clin Rheumatol* 23: 469-80

Pego-Reigosa JM, Nicholson L, Pooley N, Langham S, Embleton N, Marjenberg Z, Barut V, Desta B, Wang X, Langham J, Hammond ER (2021) The risk of infections in adult patients with systemic lupus erythematosus: systematic review and meta-analysis. *Rheumatology (Oxford)* 60: 60-72

Pellefigues C, Charles N (2013) The deleterious role of basophils in systemic lupus erythematosus. *Curr Opin Immunol* 25: 704-11

Perriard G, Mathias A, Enz L, Canales M, Schluep M, Gentner M, Schaeren-Wiemers N, Du Pasquier RA (2015) Interleukin-22 is increased in multiple sclerosis patients and targets astrocytes. *J Neuroinflammation* 12: 119

Petri M (2007) Systemic lupus erythematosus. In *Current Diagnosis and Treatment: Rheumatology*, Imboden J, Hellmann D, Stone JH (eds)

Petri M, Allbritton J (1992) Antibiotic allergy in systemic lupus erythematosus: a case-control study. *J Rheumatol* 19: 265-9

Petri M, Kawata AK, Fernandes AW, Gajria K, Greth W, Hareendran A, Ethgen D (2013) Impaired health status and the effect of pain and fatigue on functioning in clinical trial patients with systemic lupus erythematosus. *J Rheumatol* 40: 1865-74

Petri M, Orbai AM, Alarcon GS, Gordon C, Merrill JT, Fortin PR, Bruce IN, Isenberg D, Wallace DJ, Nived O, Sturfelt G, Ramsey-Goldman R, Bae SC, Hanly JG, Sanchez-Guerrero J, Clarke A, Aranow C, Manzi S, Urowitz M, Gladman D et al. (2012) Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 64: 2677-86

Petri MA, Barr E, Magder LS (2019) Development of a systemic lupus erythematosus cardiovascular risk equation. *Lupus science & medicine* 6: e000346

Piantoni S, Regola F, Zanola A, Andreoli L, Dall'Ara F, Tincani A, Airo P (2018) Effector T-cells are expanded in systemic lupus erythematosus patients with high disease activity and damage indexes. *Lupus* 27: 143-149

Pieper J, Dubnovitsky A, Gerstner C, James EA, Rieck M, Kozhukh G, Tandre K, Pellegrino S, Gebe JA, Ronnblom L, Sandalova T, Kwok WW, Klareskog L, Buckner JH, Achour A, Malmstrom V (2018) Memory T cells specific to citrullinated alpha-enolase are enriched in the rheumatic joint. *J Autoimmun* 92: 47-56

Piga M, Floris A, Cappellazzo G, Chessa E, Congia M, Mathieu A, Cauli A (2017) Failure to achieve lupus low disease activity state (LLDAS) six months after diagnosis is associated with early damage accrual in Caucasian patients with systemic lupus erythematosus. *Arthritis Res Ther* 19: 247

Piga M, Gabba A, Congia M, Figus F, Cauli A, Mathieu A (2016) Predictors of musculoskeletal flares and Jaccouds arthropathy in patients with systemic lupus erythematosus: A 5-year prospective study. *Semin Arthritis Rheum* 46: 217-224

Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Perez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW, Jr., Hammitt LL, Tureci O et al. (2020) Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 383: 2603-2615

Pope J, Jerome D, Fenlon D, Krizova A, Ouimet J (2003) Frequency of adverse drug reactions in patients with systemic lupus erythematosus. *J Rheumatol* 30: 480-4

Pordeus V, Barzilai O, Sherer Y, Luiz RR, Blank M, Bizzaro N, Villalta D, Anaya JM, Shoenfeld Y (2008) A latitudinal gradient study of common anti-infectious agent antibody prevalence in Italy and Colombia. *Isr Med Assoc J* 10: 65-8

Portales-Perez D, Alarcon-Segovia D, Llorente L, Ruiz-Arguelles A, Abud-Mendoza C, Baranda L, de la Fuente H, Ternynck T, Gonzalez-Amaro R (1998) Penetrating anti-DNA monoclonal antibodies induce activation of human peripheral blood mononuclear cells. *J Autoimmun* 11: 563-71

Preziosa P, Rocca MA, Ramirez GA, Bozzolo EP, Canti V, Pagani E, Valsasina P, Moiola L, Rovere-Querini P, Manfredi AA, Filippi M (2019) Structural and functional brain connectomes in patients with systemic lupus erythematosus. *Eur J Neurol*

Putterman C, Diamond B (1998) Immunization with a peptide surrogate for double-stranded DNA (dsDNA) induces autoantibody production and renal immunoglobulin deposition. *J Exp Med* 188: 29-38

Quercia O, Incorvaia C, Puccinelli P, Scurati S, Emiliani F, Frati F, Stefanini GF (2012) Prevalence of allergic disorders in Italy: the Cotignola population study. *Eur Ann Allergy Clin Immunol* 44: 5-11

Quiralte J, Sanchez-Garcia F, Torres MJ, Blanco C, Castillo R, Ortega N, de Castro FR, Perez-Aciego P, Carrillo T (1999) Association of HLA-DR11 with the anaphylactoid reaction caused by nonsteroidal anti-inflammatory drugs. *J Allergy Clin Immunol* 103: 685-9

Radic M, Herrmann M, van der Vlag J, Rekvig OP (2011) Regulatory and pathogenetic mechanisms of autoantibodies in SLE. *Autoimmunity* 44: 349-56

Rahman A, Isenberg DA (2008) Systemic lupus erythematosus. *N Engl J Med* 358: 929-39

Rahman P, Gladman DD, Urowitz MB, Hallett D, Tam LS (2001) Early damage as measured by the SLICC/ACR damage index is a predictor of mortality in systemic lupus erythematosus. *Lupus* 10: 93-6

Ramirez-Ortiz ZG, Prasad A, Griffith JW, Pendergraft WF, 3rd, Cowley GS, Root DE, Tai M, Luster AD, El Khoury J, Hacohen N, Means TK (2015) The receptor TREML4 amplifies TLR7-mediated signaling during antiviral responses and autoimmunity. *Nat Immunol* 16: 495-504

Ramirez GA (2018) Genetics in systemic lupus erythematosus: entering the borough of cardiovascular risk. *Ann Transl Med* 6: S14

Ramirez GA, Argolini LM, Bellocchi C, Moroni L, Della-Torre E, Farina N, Caporali RF, Beretta L, Gerosa M, Bozzolo EP, Dagna L (2021) Impact of the COVID-19 pandemic in patients with systemic lupus erythematosus throughout one year *Clin Immunol* Epub ahead of print

Ramirez GA, Asperti C, Cucca V, Yacoub MR (2021) Challenges to Vaccination against SARS-CoV-2 in Patients with Immune-Mediated Diseases. *Vaccines (Basel)* 9: 1147

Ramirez GA, Canti V, Del Rosso S, Erra R, Moiola L, Magnoni M, Bozzolo EP, Manfredi AA, Rovere-Querini P (2019a) Diagnostic performance of aPS/PT antibodies in neuropsychiatric lupus and cardiovascular complications of SLE. *Autoimmunity*: 1-7

Ramirez GA, Canti V, Moiola L, Magnoni M, Rovere-Querini P, Coletto LA, Dagna L, Manfredi AA, Bozzolo EP (2019b) Performance of SLE responder index and lupus

low disease activity state in real life: A prospective cohort study. *Int J Rheum Dis* 22: 1752-1761

Ramirez GA, Della-Torre E, Moroni L, Yacoub MR, Dagna L, group O-Cs (2021) Correspondence on 'Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort'. *Ann Rheum Dis* In Press

Ramirez GA, Efthymiou M, Isenberg DA, Cohen H (2019c) Under crossfire: thromboembolic risk in systemic lupus erythematosus. *Rheumatology (Oxford)* 58: 940-952

Ramirez GA, Gerosa M, Beretta L, Bellocchi C, Argolini LM, Moroni L, Della Torre E, Artusi C, Nicolosi S, Caporali R, Bozzolo EP, Dagna L, Smile MLC (2020) COVID-19 in systemic lupus erythematosus: Data from a survey on 417 patients. *Semin Arthritis Rheum* 50: 1150-1157

Ramirez GA, Lanzani C, Bozzolo EP, Citterio L, Zagato L, Casamassima N, Canti V, Sabbadini MG, Rovere-Querini P, Manunta P, Manfredi AA (2015a) TRPC6 gene variants and neuropsychiatric lupus. *J Neuroimmunol* 288: 21-4

Ramirez GA, Lanzani C, Bozzolo EP, Zagato L, Citterio L, Casamassima N, Canti V, Sabbadini MG, Rovere-Querini P, Manunta P, Manfredi AA (2015b) Beta-adducin and sodium-calcium exchanger 1 gene variants are associated with systemic lupus erythematosus and lupus nephritis. *Rheumatol Int*

Ramirez GA, Moroni L, Della-Torre E, Gerosa M, Beretta L, Bozzolo EP, Dagna L (2020) Systemic lupus erythematosus and COVID-19: what we know so far. *Ann Rheum Dis*

Ramirez GA, Rocca MA, Preziosa P, Bozzolo EP, Pagani E, Canti V, Moiola L, Rovere-Querini P, Manfredi AA, Filippi M (2021) Quantitative MRI adds to neuropsychiatric lupus diagnostics. *Rheumatology (Oxford)* 60: 3278-3288

Ramirez GA, Tejera-Segura B, Lopez R, Rahman A, Isenberg DA (2018) 138 Disease trends and phenotypes among different age groups: a study in lupus. *Rheumatology (Oxford)* 57: key075. 362

Reddy V, Cambridge G, Isenberg DA, Glennie MJ, Cragg MS, Leandro M (2015) Internalization of rituximab and the efficiency of B Cell depletion in rheumatoid arthritis and systemic lupus erythematosus. *Arthritis & rheumatology* 67: 2046-55

Reddy V, Croca S, Gerona D, De La Torre I, Isenberg D, McDonald V, Leandro M, Cambridge G (2013) Serum rituximab levels and efficiency of B cell depletion: differences between patients with rheumatoid arthritis and systemic lupus erythematosus. *Rheumatology (Oxford)* 52: 951-2

Reddy V, Jayne D, Close D, Isenberg D (2013) B-cell depletion in SLE: clinical and trial experience with rituximab and ocrelizumab and implications for study design. *Arthritis Res Ther* 15 Suppl 1: S2

Rendine S, Borelli I, Barbanti M, Sacchi N, Roggero S, Curtoni ES (1998) HLA polymorphisms in Italian bone marrow donors: a regional analysis. *Tissue Antigens* 52: 135-46

Rider JR, Ollier WE, Lock RJ, Brookes ST, Pamphilon DH (1997) Human cytomegalovirus infection and systemic lupus erythematosus. *Clin Exp Rheumatol* 15: 405-9

Robinson S, Thomas R (2021) Potential for Antigen-Specific Tolerizing Immunotherapy in Systematic Lupus Erythematosus. *Front Immunol* 12: 654701

Rother N, van der Vlag J (2015) Disturbed T Cell Signaling and Altered Th17 and Regulatory T Cell Subsets in the Pathogenesis of Systemic Lupus Erythematosus. *Front Immunol* 6: 610

Rovere Querini P, De Lorenzo R, Conte C, Brioni E, Lanzani C, Yacoub MR, Chionna R, Martinenghi S, Vitali G, Tresoldi M, Ciceri F (2020) Post-COVID-19 follow-up clinic: depicting chronicity of a new disease. *Acta Biomed* 91: 22-28

Rovin BH, Furie R, Latinis K, Looney RJ, Fervenza FC, Sanchez-Guerrero J, Maciuca R, Zhang D, Garg JP, Brunetta P, Appel G, Group LI (2012) Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum* 64: 1215-26

Rua-Figueroa I, Lopez-Longo J, Galindo-Izquierdo M, Calvo-Alen J, Del Campo V, Olive-Marques A, Perez-Vicente S, Fernandez-Nebro A, Andres M, Erausquin C, Tomero E, Horcada L, Uriarte E, Freire M, Montilla C, Sanchez-Atrio A, Santos G, Boteanu A, Diez-Alvarez E, Narvaez J et al. (2017) Incidence, associated factors and clinical impact of severe infections in a large, multicentric cohort of patients with systemic lupus erythematosus. *Semin Arthritis Rheum* 47: 38-45

Ruiz-Irastorza G, Olivares N, Ruiz-Arruza I, Martinez-Berriotxoa A, Egurbide MV, Aguirre C (2009) Predictors of major infections in systemic lupus erythematosus. *Arthritis Res Ther* 11: R109

Ruterbusch M, Pruner KB, Shehata L, Pepper M (2020) In Vivo CD4(+) T Cell Differentiation and Function: Revisiting the Th1/Th2 Paradigm. *Annu Rev Immunol* 38: 705-725

Saini C, Ramesh V, Nath I (2013) CD4+ Th17 cells discriminate clinical types and constitute a third subset of non Th1, Non Th2 T cells in human leprosy. *PLoS Negl Trop Dis* 7: e2338

Salazar-Camarena DC, Ortiz-Lazareno PC, Cruz A, Oregon-Romero E, Machado-Contreras JR, Munoz-Valle JF, Orozco-Lopez M, Marin-Rosales M, Palafox-Sanchez CA (2016) Association of BAFF, APRIL serum levels, BAFF-R, TACI and BCMA expression on peripheral B-cell subsets with clinical manifestations in systemic lupus erythematosus. *Lupus* 25: 582-92

Salem D, Subang R, Kuwana M, Levine JS, Rauch J (2018) T cells from induced and spontaneous models of SLE recognize a common T cell epitope on beta2-glycoprotein I. *Cell Mol Immunol*

Salem D, Subang R, Okazaki Y, Laplante P, Levine JS, Kuwana M, Rauch J (2015) beta2-Glycoprotein I-specific T cells are associated with epitope spread to lupus-related autoantibodies. *J Biol Chem* 290: 5543-55

Sallusto F (2016) Heterogeneity of Human CD4(+) T Cells Against Microbes. *Annu Rev Immunol* 34: 317-34

Sander DM, Szabo S, Gallaher WR, Deas JE, Thompson JJ, Cao Y, Luo-Zhang H, Liu LG, Colmegna I, Koehler J, Espinoza LR, Alexander SS, Hart DJ, Tom DM, Fermin CD, Jaspan JJ, Kulakosky PC, Tenenbaum SA, Wilson RB, Garry RF (2005) Involvement of human intracisternal A-type retroviral particles in autoimmunity. *Microsc Res Tech* 68: 222-34

Sarbu N, Alobeidi F, Toledano P, Espinosa G, Giles I, Rahman A, Yousry T, Capurro S, Jager R, Cervera R, Bargallo N (2015) Brain abnormalities in newly diagnosed neuropsychiatric lupus: systematic MRI approach and correlation with clinical and laboratory data in a large multicenter cohort. *Autoimmunity reviews* 14: 153-9

Sarbu N, Toledano P, Calvo A, Roura E, Sarbu MI, Espinosa G, Llado X, Cervera R, Bargallo N (2017) Advanced MRI techniques: biomarkers in neuropsychiatric lupus. *Lupus* 26: 510-516

Sasidhar MV, Itoh N, Gold SM, Lawson GW, Voskuhl RR (2012) The XX sex chromosome complement in mice is associated with increased spontaneous lupus compared with XY. *Ann Rheum Dis* 71: 1418-22

Sawada T, Fujimori D, Yamamoto Y (2019) Systemic lupus erythematosus and immunodeficiency. *Immunol Med* 42: 1-9

Sawalha AH, Zhao M, Coit P, Lu Q (2020) Epigenetic dysregulation of ACE2 and interferon-regulated genes might suggest increased COVID-19 susceptibility and severity in lupus patients. *Clin Immunol* 215: 108410

Scalzi LV, Hollenbeak CS, Wang L (2010) Racial disparities in age at time of cardiovascular events and cardiovascular-related death in patients with systemic lupus erythematosus. *Arthritis Rheum* 62: 2767-75

Scapini P, Hu Y, Chu CL, Migone TS, Defranco AL, Cassatella MA, Lowell CA (2010) Myeloid cells, BAFF, and IFN-gamma establish an inflammatory loop that exacerbates autoimmunity in Lyn-deficient mice. *J Exp Med* 207: 1757-73

Schett G, Smole J, Zimmermann C, Hiesberger H, Hoefler E, Fournel S, Muller S, Rubin RL, Steiner G (2002) The autoimmune response to chromatin antigens in systemic lupus erythematosus: autoantibodies against histone H1 are a highly specific marker for SLE associated with increased disease activity. *Lupus* 11: 704-15

Sciascia S, Sanna G, Murru V, Roccatello D, Khamashta MA, Bertolaccini ML (2013) GAPSS: the Global Anti-Phospholipid Syndrome Score. *Rheumatology (Oxford)* 52: 1397-403

Scire CA, Carrara G, Zanetti A, Landolfi G, Chighizola C, Alunno A, Andreoli L, Caporali R, Gerli R, Sebastiani GD, Valesini G, Sinigaglia L, Italian Registry of the Italian Society for R (2020) COVID-19 in rheumatic diseases in Italy: first results from the Italian registry of the Italian Society for Rheumatology (CONTROL-19). *Clin Exp Rheumatol* 38: 748-753

Scofield L, Reinlib L, Alarcon GS, Cooper GS (2008) Employment and disability issues in systemic lupus erythematosus: a review. *Arthritis Rheum* 59: 1475-9

Scornet N, Delarue-Cochin S, Azoury ME, Le Mignon M, Chemelle JA, Nony E, Maillere B, Terreux R, Pallardy M, Joseph D (2016) Bioinspired Design and Oriented Synthesis of Immunogenic Site-Specifically Penicilloylated Peptides. *Bioconjug Chem* 27: 2629-2645

Sekigawa I, Yoshiike T, Iida N, Hashimoto H, Ogawa H (2002) Allergic disorders in systemic lupus erythematosus: prevalence and family history. *Lupus* 11: 426-9

Sen Y, Chunsong H, Baojun H, Linjie Z, Qun L, San J, Qiuping Z, Junyan L, Zhang X, Jinquan T (2004) Aberration of CCR7 CD8 memory T cells from patients with systemic lupus erythematosus: an inducer of T helper type 2 bias of CD4 T cells. *Immunology* 112: 274-89

Sequeira JF, Cesic D, Keser G, Bukelica M, Karanagnostis S, Khamashta MA, Hughes GR (1993) Allergic disorders in systemic lupus erythematosus. *Lupus* 2: 187-91

Serra P, Santamaria P (2020) Peptide-MHC-Based Nanomedicines for the Treatment of Autoimmunity: Engineering, Mechanisms, and Diseases. *Front Immunol* 11: 621774

Shah K, Lee WW, Lee SH, Kim SH, Kang SW, Craft J, Kang I (2010) Dysregulated balance of Th17 and Th1 cells in systemic lupus erythematosus. *Arthritis Res Ther* 12: R53

Shahar E, Lorber M (1997) Allergy and SLE: common and variable. *Isr J Med Sci* 33: 147-9

Shapira Y, Poratkatz BS, Gilburd B, Barzilai O, Ram M, Blank M, Lindeberg S, Frostegard J, Anaya JM, Bizzaro N, Jara LJ, Damoiseaux J, Shoenfeld Y, Levin NA (2012) Geographical differences in autoantibodies and anti-infectious agents antibodies among healthy adults. *Clin Rev Allergy Immunol* 42: 154-63

Sharma BK, Jain S, Suri S, Numano F (1996) Diagnostic criteria for Takayasu arteritis. *Int J Cardiol* 54 Suppl: S141-7

Shen TC, Tu CY, Lin CL, Wei CC, Li YF (2014) Increased risk of asthma in patients with systemic lupus erythematosus. *Am J Respir Crit Care Med* 189: 496-9

Shimane K, Kochi Y, Suzuki A, Okada Y, Ishii T, Horita T, Saito K, Okamoto A, Nishimoto N, Myouzen K, Kubo M, Hirakata M, Sumida T, Takasaki Y, Yamada R, Nakamura Y, Kamatani N, Yamamoto K (2013) An association analysis of HLA-DRB1 with systemic lupus erythematosus and rheumatoid arthritis in a Japanese population: effects of *09:01 allele on disease phenotypes. *Rheumatology (Oxford)* 52: 1172-82

Shin HD, Park BL, Kim LH, Lee HS, Kim TY, Bae SC (2004) Common DNase I polymorphism associated with autoantibody production among systemic lupus erythematosus patients. *Hum Mol Genet* 13: 2343-50

Sibbitt WL, Jr., Brooks WM, Kornfeld M, Hart BL, Bankhurst AD, Roldan CA (2010) Magnetic resonance imaging and brain histopathology in neuropsychiatric systemic lupus erythematosus. *Semin Arthritis Rheum* 40: 32-52

Silva LM, Garcia AB, Donadi EA (2002) Increased lymphocyte death by neglect-apoptosis is associated with lymphopenia and autoantibodies in lupus patients presenting with neuropsychiatric manifestations. *J Neurol* 249: 1048-1054

Simard JF, Costenbader KH (2015) Epidemiology and classification of systemic lupus erythemato. In *Rheumatology, VI Edition*, Hochberg MC (ed) Mosby

Snir O, Backlund J, Bostrom J, Andersson I, Kihlberg J, Buckner JH, Klareskog L, Holmdahl R, Malmstrom V (2012) Multifunctional T cell reactivity with native and glycosylated type II collagen in rheumatoid arthritis. *Arthritis Rheum* 64: 2482-8

Sontheimer RD (2004) Skin manifestations of systemic autoimmune connective tissue disease: diagnostics and therapeutics. *Best Pract Res Clin Rheumatol* 18: 429-62

Souliotis VL, Sfikakis PP (2015) Increased DNA double-strand breaks and enhanced apoptosis in patients with lupus nephritis. *Lupus* 24: 804-15

Staats J, Divekar A, McCoy JP, Jr., Maecker HT (2019) Guidelines for Gating Flow Cytometry Data for Immunological Assays. *Methods Mol Biol* 2032: 81-104

Stanley AC, Lacy P (2010) Pathways for cytokine secretion. *Physiology (Bethesda)* 25: 218-29

Starshinova A, Dovgalyuk I, Berkos A, Ovchinnikova Y, Bubnova L, Yablonskiy P (2018) The effect of human leukocyte Antigens-DRB1 alleles on development of different tuberculosis forms in children. *Int J Mycobacteriol* 7: 117-121

Stevens DM, Crist RM, Stern ST (2020) Nanomedicine Reformulation of Chloroquine and Hydroxychloroquine. *Molecules* 26

Stohl W (2014) Therapeutic targeting of the BAFF/APRIL axis in systemic lupus erythematosus. *Expert Opin Ther Targets* 18: 473-89

Stoll T, Seifert B, Isenberg DA (1996) SLICC/ACR Damage Index is valid, and renal and pulmonary organ scores are predictors of severe outcome in patients with systemic lupus erythematosus. *Br J Rheumatol* 35: 248-54

Strangfeld A, Schafer M, Gianfrancesco MA, Lawson-Tovey S, Liew JW, Ljung L, Mateus EF, Richez C, Santos MJ, Schmajuk G, Scire CA, Sirotich E, Sparks JA, Sufka P, Thomas T, Trupin L, Wallace ZS, Al-Adely S, Bachiller-Corral J, Bhana S et al. (2021) Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann Rheum Dis*

Strom BL, Reidenberg MM, West S, Snyder ES, Freundlich B, Stolley PD (1994) Shingles, allergies, family medical history, oral contraceptives, and other potential risk factors for systemic lupus erythematosus. *Am J Epidemiol* 140: 632-42

Strom BL, Schinnar R, Apter AJ, Margolis DJ, Lautenbach E, Hennessy S, Bilker WB, Pettitt D (2003) Absence of cross-reactivity between sulfonamide antibiotics and sulfonamide nonantibiotics. *N Engl J Med* 349: 1628-35

Strufaldi FL, Menezes Neves P, Dias CB, Yu L, Woronik V, Cavalcante LB, Malheiros D, Jorge LB (2021) Renal thrombotic microangiopathy associated to worse renal prognosis in Lupus Nephritis. *J Nephrol* 34: 1147-1156

Summers S, Odobasic D, Khouri M, Steinmetz O, Yang Y, Holdsworth S, Kitching A (2014) Endogenous interleukin (IL)-17A promotes pristane-induced systemic autoimmunity and lupus nephritis induced by pristane. *Clin Exp Immunol* 176: 341-350

Swiecki M, Colonna M (2015) The multifaceted biology of plasmacytoid dendritic cells. *Nat Rev Immunol* 15: 471-85

Talken BL, Schafermeyer KR, Bailey CW, Lee DR, Hoffman RW (2001) T cell epitope mapping of the Smith antigen reveals that highly conserved Smith antigen motifs are the dominant target of T cell immunity in systemic lupus erythematosus. *J Immunol* 167: 562-8

Tang S, Zhang Y, Yin S, Gao X, Shi W, Wang Y, Huang X, Wang L, Zou L, Zhao J, Huang Y, Shan L, Gounni AS, Wu Y, Zhang J (2015) Neutrophil extracellular trap formation is associated with autophagy-related signaling in ANCA-associated vasculitis. *Clin Exp Immunol*

Tani C, Carli L, Stagnaro C, Elefante E, Signorini V, Balestri F, Delle Sedie A, Mosca M (2018) Imaging of joints in systemic lupus erythematosus. *Clin Exp Rheumatol* 36 Suppl 114: 68-73

Tani C, Elefante E, Signorini V, Zucchi D, Lorenzoni V, Carli L, Stagnaro C, Ferro F, Mosca M (2019) Glucocorticoid withdrawal in systemic lupus erythematosus: are remission and low disease activity reliable starting points for stopping treatment? A real-life experience. *RMD Open* 5: e000916

Tanwani J, Tselios K, Gladman DD, Su J, Urowitz MB (2018) Lupus myocarditis: a single center experience and a comparative analysis of observational cohort studies. *Lupus* 27: 1296-1302

Tejedor Alonso MA, Moro Moro M, Mugica Garcia MV, Esteban Hernandez J, Rosado Ingelmo A, Vila Albelda C, Gomez Traseira C, Cardenas Contreras R, Sanz Sacristan J, Hernandez Merino A (2012) Incidence of anaphylaxis in the city of Alcorcon (Spain): a population-based study. *Clin Exp Allergy* 42: 578-89

Terao C, Yamada R, Mimori T, Yamamoto K, Sumida T (2014) A nationwide study of SLE in Japanese identified subgroups of patients with clear signs patterns and associations between signs and age or sex. *Lupus* 23: 1435-42

Terrier B, Amoura Z, Ravaud P, Hachulla E, Jouenne R, Combe B, Bonnet C, Cacoub P, Cantagrel A, de Bandt M, Fain O, Fautrel B, Gaudin P, Godeau B, Harle JR, Hot A, Kahn JE, Lambotte O, Larroche C, Leone J et al. (2010) Safety and efficacy of rituximab in systemic lupus erythematosus: results from 136 patients from the French AutoImmunity and Rituximab registry. *Arthritis Rheum* 62: 2458-66

Teruel M, Alarcon-Riquelme ME (2016a) The genetic basis of systemic lupus erythematosus: What are the risk factors and what have we learned. *J Autoimmun* 74: 161-175

Teruel M, Alarcon-Riquelme ME (2016b) Genetics of systemic lupus erythematosus and Sjogren's syndrome: an update. *Curr Opin Rheumatol* 28: 506-14

Tesch S, Abdirama D, Griessbach AS, Brand HA, Goerlich N, Humrich JY, Bacher P, Hiepe F, Riemekasten G, Enghard P (2020) Identification and characterization of antigen-specific CD4(+) T cells targeting renally expressed antigens in human lupus nephritis with two independent methods. *Sci Rep* 10: 21312

Testi R, Phillips JH, Lanier LL (1989) T cell activation via Leu-23 (CD69). *J Immunol* 143: 1123-8

Touma Z, Gladman DD, Su J, Anderson N, Urowitz MB (2018) A novel lupus activity index accounting for glucocorticoids: SLEDAI-2K glucocorticoid index. *Rheumatology (Oxford)* 57: 1370-1376

Touma Z, Gladman DD, Urowitz MB (2013) Vaccination and auto-immune rheumatic diseases: lessons learnt from the 2009 H1N1 influenza virus vaccination campaign. *Curr Opin Rheumatol* 25: 164-70

Trela M, Nelson PN, Rylance PB (2016) The role of molecular mimicry and other factors in the association of Human Endogenous Retroviruses and autoimmunity. *APMIS* 124: 88-104

Tsang ASMWP, Bultink IEM, Korswagen LA, van der Horst A, Rensink I, de Boer M, Hamann D, Voskuyl AE, Wouters D (2017) Comprehensive approach to study complement C4 in systemic lupus erythematosus: Gene polymorphisms, protein levels and functional activity. *Mol Immunol* 92: 125-131

Tsokos GC (2011) Systemic lupus erythematosus. *N Engl J Med* 365: 2110-21

Tsokos GC, Magrath IT, Balow JE (1983) Epstein-Barr virus induces normal B cell responses but defective suppressor T cell responses in patients with systemic lupus erythematosus. *J Immunol* 131: 1797-801

Tummala R, Abreu G, Pineda L, Michaels MA, Kalyani RN, Furie RA, Morand EF (2021) Safety profile of anifrolumab in patients with active SLE: an integrated analysis of phase II and III trials. *Lupus science & medicine* 8

Tyden H, Lood C, Gullstrand B, Nielsen CT, Heegaard NHH, Kahn R, Jonsen A, Bengtsson AA (2017) Endothelial dysfunction is associated with activation of the type I interferon system and platelets in patients with systemic lupus erythematosus. *RMD Open* 3: e000508

Uchtenhagen H, Rims C, Blahnik G, Chow IT, Kwok WW, Buckner JH, James EA (2016) Efficient ex vivo analysis of CD4+ T-cell responses using combinatorial HLA class II tetramer staining. *Nat Commun* 7: 12614

Ugarte-Gil MF, Pons-Estel GJ, Molineros J, Wojdyla D, McGwin G, Jr., Nath SK, Pons-Estel BA, Alarcon-Riquelme M, Alarcon GS (2016) Disease features and outcomes in United States lupus patients of Hispanic origin and their Mestizo counterparts in Latin America: a commentary. *Rheumatology (Oxford)* 55: 436-40

Unterman A, Nolte JE, Boaz M, Abady M, Shoenfeld Y, Zandman-Goddard G (2011) Neuropsychiatric syndromes in systemic lupus erythematosus: a meta-analysis. *Semin Arthritis Rheum* 41: 1-11

Urowitz MB, Ohsfeldt RL, Wielage RC, Kelton KA, Asukai Y, Ramachandran S (2019) Organ damage in patients treated with belimumab versus standard of care: a propensity score-matched comparative analysis. *Ann Rheum Dis*

van Vollenhoven R, Voskuyl A, Bertsias G, Aranow C, Aringer M, Arnaud L, Askanase A, Balazova P, Bonfa E, Bootsma H, Boumpas D, Bruce I, Cervera R, Clarke A, Coney C, Costedoat-Chalumeau N, Czirjak L, Derksen R, Doria A, Dorner T et al. (2017) A framework for remission in SLE: consensus findings from a large international task force on definitions of remission in SLE (DORIS). *Ann Rheum Dis* 76: 554-561

van Vollenhoven RF, Hahn BH, Tsokos GC, Wagner CL, Lipsky P, Touma Z, Werth VP, Gordon RM, Zhou B, Hsu B, Chevrier M, Triebel M, Jordan JL, Rose S (2018) Efficacy and safety of ustekinumab, an IL-12 and IL-23 inhibitor, in patients with active systemic lupus erythematosus: results of a multicentre, double-blind, phase 2, randomised, controlled study. *Lancet* 392: 1330-1339

Vaughn SE, Kottyan LC, Munroe ME, Harley JB (2012) Genetic susceptibility to lupus: the biological basis of genetic risk found in B cell signaling pathways. *J Leukoc Biol* 92: 577-91

Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, Rubin CJ, Zhao W, Olsen SH, Klinker M, Shealy D, Denny MF, Plumas J, Chaperot L, Kretzler M, Bruce AT, Kaplan MJ (2011) Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *J Immunol* 187: 538-52

Vincent FB, Northcott M, Hoi A, Mackay F, Morand EF (2013) Clinical associations of serum interleukin-17 in systemic lupus erythematosus. *Arthritis Res Ther* 15: R97

Vivero F, Gonzalez-Echavarri C, Ruiz-Estevez B, Maderuelo I, Ruiz-Irastorza G (2016) Prevalence and predictors of valvular heart disease in patients with systemic lupus erythematosus. *Autoimmunity reviews* 15: 1134-1140

Walker ME, Hatfield JK, Brown MA (2012) New insights into the role of mast cells in autoimmunity: evidence for a common mechanism of action? *Biochim Biophys Acta* 1822: 57-65

Wallace D (2019) A 52-Week, Randomized, Double-Blind, Parallel-Group, Placebo-Controlled Study to Evaluate the Efficacy and Safety of a 200-mcg Dose of IPP-201101 Plus Standard of Care in Patients With Systemic Lupus Erythematosus - https://www.clinicaltrials.gov/ct2/show/results/NCT02504645. In Clinicaltrials.gov:

Wallace DJ, Furie RA, Tanaka Y, Kalunian KC, Mosca M, Petri MA, Dorner T, Cardiel MH, Bruce IN, Gomez E, Carmack T, DeLozier AM, Janes JM, Linnik MD, de Bono S, Silk ME, Hoffman RW (2018) Baricitinib for systemic lupus erythematosus: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet* 392: 222-231

Wallace DJ, Kalunian K, Petri MA, Strand V, Houssiau FA, Pike M, Kilgallen B, Bongardt S, Barry A, Kelley L, Gordon C (2014) Efficacy and safety of epratuzumab in patients with moderate/severe active systemic lupus erythematosus: results from EMBLEM, a phase IIb, randomised, double-blind, placebo-controlled, multicentre study. *Ann Rheum Dis* 73: 183-90

Wambre E, DeLong JH, James EA, LaFond RE, Robinson D, Kwok WW (2012) Differentiation stage determines pathologic and protective allergen-specific CD4+ T-cell outcomes during specific immunotherapy. *J Allergy Clin Immunol* 129: 544-51, 551 e1-7

Wang X, Hefton A, Ni K, Ukadike KC, Bowen MA, Eckert M, Stevens A, Lood C, Mustelin T (2021) Autoantibodies Against Unmodified and Citrullinated Human Endogenous Retrovirus K Envelope Protein in Patients With Rheumatoid Arthritis. *J Rheumatol*

Wang YF, Zhang Y, Lin Z, Zhang H, Wang TY, Cao Y, Morris DL, Sheng Y, Yin X, Zhong SL, Gu X, Lei Y, He J, Wu Q, Shen JJ, Yang J, Lam TH, Lin JH, Mai ZM, Guo M et al. (2021) Identification of 38 novel loci for systemic lupus erythematosus and genetic heterogeneity between ancestral groups. *Nat Commun* 12: 772

Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB, Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM et al. (2004) The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 65: 521-30

Wen Z, Shen Y, Berry G, Shahram F, Li Y, Watanabe R, Liao YJ, Goronzy JJ, Weyand CM (2017) The microvascular niche instructs T cells in large vessel vasculitis via the VEGF-Jagged1-Notch pathway. *Sci Transl Med* 9

Weyand CM, Schonberger J, Oppitz U, Hunder NN, Hicok KC, Goronzy JJ (1994) Distinct vascular lesions in giant cell arteritis share identical T cell clonotypes. *J Exp Med* 179: 951-60

Wincup C, Sawford N, Rahman A (2021) Pathological mechanisms of abnormal iron metabolism and mitochondrial dysfunction in systemic lupus erythematosus. *Expert Rev Clin Immunol* 17: 957-967

Wiseman SJ, Bastin ME, Hamilton IF, Hunt D, Ritchie SJ, Amft EN, Thomson S, Belch JF, Ralston SH, Wardlaw JM (2017) Fatigue and cognitive function in systemic lupus erythematosus: associations with white matter microstructural damage. A diffusion tensor MRI study and meta-analysis. *Lupus* 26: 588-597

Wong A, Seger DL, Lai KH, Goss FR, Blumenthal KG, Zhou L (2019) Drug Hypersensitivity Reactions Documented in Electronic Health Records within a Large Health System. *J Allergy Clin Immunol Pract* 7: 1253-1260 e3

Wozniacka A, Sysa-Jedrzejowska A, Robak E, Samochocki Z, Zak-Prelich M (2003) Allergic diseases, drug adverse reactions and total immunoglobulin E levels in lupus erythematosus patients. *Mediators Inflamm* 12: 95-9

Wu YF, Wang LY, Lee TD, Lin HH, Hu CT, Cheng ML, Lo SY (2004) HLA phenotypes and outcomes of hepatitis B virus infection in Taiwan. *J Med Virol* 72: 17-25

Xiaoyan Z, Pirskanen R, Malmstrom V, Lefvert AK (2006) Expression of OX40 (CD134) on CD4+ T-cells from patients with myasthenia gravis. *Clin Exp Immunol* 143: 110-6

Yang J, Chu Y, Yang X, Gao D, Zhu L, Yang X, Wan L, Li M (2009) Th17 and natural Treg cell population dynamics in systemic lupus erythematosus. *Arthritis Rheum* 60: 1472-83

Yang X, Yang J, Chu Y, Xue Y, Xuan D, Zheng S, Zou H (2014) T follicular helper cells and regulatory B cells dynamics in systemic lupus erythematosus. *PLoS One* 9: e88441

Yee CS, Cresswell L, Farewell V, Rahman A, Teh LS, Griffiths B, Bruce IN, Ahmad Y, Prabu A, Akil M, McHugh N, D'Cruz D, Khamashta MA, Isenberg DA, Gordon C (2010) Numerical scoring for the BILAG-2004 index. *Rheumatology (Oxford)* 49: 1665-9

Yeste A, Nadeau M, Burns EJ, Weiner HL, Quintana FJ (2012) Nanoparticle-mediated codelivery of myelin antigen and a tolerogenic small molecule suppresses experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 109: 11270-5

Zabinska M, Krajewska M, Koscielska-Kasprzak K, Klinger M (2016) CD3(+)CD8(+)CD28(-) T Lymphocytes in Patients with Lupus Nephritis. *Journal of immunology research* 2016: 1058165

Zannikou M, Bellou S, Eliades P, Hatzioannou A, Mantzaris MD, Carayanniotis G, Avrameas S, Lymberi P (2016) DNA–histone complexes as ligands amplify cell penetration and nuclear targeting of anti-DNA antibodies via energy-independent mechanisms. *Immunology* 147: 73-81

Zen M, Iaccarino L, Gatto M, Saccon F, Larosa M, Ghirardello A, Punzi L, Doria A (2018) Lupus low disease activity state is associated with a decrease in damage progression in Caucasian patients with SLE, but overlaps with remission. *Ann Rheum Dis* 77: 104-110

Zhang L, Bertucci AM, Ramsey-Goldman R, Harsha-Strong ER, Burt RK, Datta SK (2013) Major pathogenic steps in human lupus can be effectively suppressed by nucleosomal histone peptide epitope-induced regulatory immunity. *Clin Immunol* 149: 365-78

Zhang SX, Ma XW, Li YF, Lai NL, Huang ZH, Fan K, Wang CH, Li XF (2018) The Proportion of Regulatory T Cells in Patients with Systemic Lupus Erythematosus: A Meta-Analysis. *Journal of immunology research* 2018: 7103219

Zhao L, Li Y, Smith Jr M, Wang J, Zhang W, Tang F, Tian X, Wang H, Zhang F, Ba D (2010) Expressions of BAFF/BAFF receptors and their correlation with disease activity in Chinese SLE patients. *Lupus* 19: 1534-1549

Zhao Y, Zhao Y, Zhang Y, Zhang L (2019a) HLA-II genes are associated with outcomes of specific immunotherapy for allergic rhinitis. *Int Forum Allergy Rhinol* 9: 1311-1317

Zhao Z, Ren J, Dai C, Kannapell CC, Wang H, Gaskin F, Fu SM (2019b) Nature of T cell epitopes in lupus antigens and HLA-DR determines autoantibody initiation and diversification. *Ann Rheum Dis* 78: 380-390

Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, Ouyang W (2007) Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 445: 648-51

Zhong W, Jiang Z, Wu J, Jiang Y, Zhao L (2018) CCR6(+) Th cell distribution differentiates systemic lupus erythematosus patients based on anti-dsDNA antibody status. *PeerJ* 6: e4294

Zhou H, Hu B, Huang N, Mo X, Li W, Zhang B, Wei B, Gao M, Wang Y, Liu X, Liao J (2018) Aberrant T cell subsets and cytokines expression profile in systemic lupus erythematosus. *Clin Rheumatol* 37: 2405-2413

Zimmer R, Scherbarth HR, Rillo OL, Gomez-Reino JJ, Muller S (2013) Lupuzor/P140 peptide in patients with systemic lupus erythematosus: a randomised, double-blind, placebo-controlled phase IIb clinical trial. *Ann Rheum Dis* 72: 1830-5

Zonana-Nacach A, Camargo-Coronel A, Yanez P, Sanchez L, Jimenez-Balderas FJ, Fraga A (2001) Infections in outpatients with systemic lupus erythematosus: a prospective study. *Lupus* 10: 505-10

APPENDICES

Appendix 1: prevalence of allergy and asthma in the general population according to different data sources

Source	Year	Region	Method	Allergy	Allergy excl. drug allergy	Drug Allergy	Allergy excluding asthma	Asthma
Eurostat	2014	EU	Simplified questionnaire	ND	ND	ND	16.9%	5.9%
Eurostat	2014	Italy	Simplified questionnaire	ND	ND	ND	15.2%	4.9%
Eurostat	2014	Sweden	Simplified questionnaire	ND	ND	ND	22.7%	7.8%
ISTAT	2017	Italy	Simplified questionnaire	10.7%	ND	ND	ND	ND
(Quercia et al., 2012)	ND	Italy	Specific questionnaire	ND	ND	ND	ND	6.1%
	ND	Italy	Survey on physician-confirmed diagnosis	ND	16.5%*	ND	ND	3.6%
(Wong et al., 2019)	2000-2013	USA	Supervised ICD-9-based analysis	ND	ND	13.8%	ND	ND
(Langen et al., 2013)	2008-2011	Germany	Survey on physician-confirmed diagnosis	ND	30%	ND	ND	8.6%

*Data kindly provided by Prof. Cristoforo Incorvaia in addition to those reported in the original manuscript.

Abbreviations. Eurostat: European Institute of Statistics; ICD: International Classification of Diseases: ISTAT: Istituto Nazionale di Statistica (Italian National Institute for Statistics); ND: no data.

Appendix 2: complete high-resolution HLA-DRB1 genotype frequency in patients, controls, and in the general population

	SLE		TAK		HC		Italian General Population*	
	%	Sample size	%	Sample size	%	Sample size	%	Sample size
DRB1*03:01	33%	185	11%	80	17%	86	19%	975
DRB1*07:01	22%	185	31%	80	22%	86	25%	975
DRB1*11:01	15%	185	19%	80	15%	86	31%	4,575
DRB1*15:01	14%	185	8%	80	13%	86	9%	4,575
DRB1*01:01	14%	185	6%	80	10%	86	14%	4,575
DRB1*14:01	12%	185	8%	80	14%	86	5%	975
DRB1*16:01	12%	185	13%	80	6%	86	10%	975
DRB1*11:04	11%	185	16%	80	14%	86	28%	4,575
DRB1*13:02	10%	185	10%	80	10%	86	9%	4,575
DRB1*13:01	7%	185	10%	80	10%	86	11%	4,575
DRB1*04:01	4%	185	8%	80	7%	86	3%	975
DRB1*08:01	3%	185	4%	80	2%	86	4%	975
DRB1*15:02	3%	185	10%	80	3%	86	2%	4,575
DRB1*04:04	3%	185	1%	80	2%	86	2%	975
DRB1*11:03	3%	185	5%	80	3%	86	3%	975
DRB1*13:03	3%	185	4%	80	1%	86	3%	975
DRB1*04:02	3%	185	4%	80	6%	86	3%	975
DRB1*10:01	2%	185	3%	80	5%	86	4%	975
DRB1*04:03	2%	185	3%	80	6%	86	3%	975
DRB1*01:03	2%	185	0%	80	2%	86	1%	4,575
DRB1*04:05	2%	185	5%	80	2%	86	3%	975
DRB1*08:04	2%	185	1%	80	0%	86	0%	975
DRB1*01:02	2%	185	9%	80	6%	86	4%	4,575
DRB1*14:04	1%	185	1%	80	0%	86	1%	975
DRB1*16:02	1%	185	1%	80	1%	86	1%	975
DRB1*09:01	1%	185	0%	80	1%	86	1%	975
DRB1*13:05	1%	185	0%	80	3%	86	1%	975
DRB1*13:04	1%	185	0%	80	0%	86	0%	975
DRB1*04:07	1%	185	0%	80	1%	86	1%	975
DRB1*12:01	1%	185	4%	80	3%	86	2%	975
DRB1*04:10	1%	185	0%	80	0%	86	0%	975
DRB1*11:02	1%	185	0%	80	1%	86	1%	975
DRB1*08:02	1%	185	0%	80	0%	86	0%	975

*Data were retrieved from the Allele Frequency Net - http://www.allelefrequencies.net - (Gonzalez-Galarza et al., 2019), based on data from multiple studies (De Re et al., 2010, Rendine et al., 1998). ND= No data available.

Appendix 3: HLA-DRB1 genotype and clinical features of patients with SLE and COVID-19

#	Age (years)	Sex	Disease duration (years)	HLA-DRB1 genotype	Main SLE features	COVID-19 course
1	56	M	23	*01:01, *16:01	Nephritis, myopericarditis, leukopenia, thrombocytopenia, arthritis, positive ADNA, low complement	Mild respiratory symptoms. Post-COVID-19 myocardial infarction
2	57	F	21	*03:01, *15:01	Serositis, arthritis, Kikuchi-Fujimoto's disease, positive ADNA.	Moderate respiratory, GI and systemic symptoms. Persistent cough.
3	53	F	28	*11:01, *14:01P	Skin manifestations, arthritis, positive ADNA, low complement	Mild respiratory symptoms.
4	41	F	9	*03:01, *13:01	Serositis, arthritis, polyadenomegaly, positive ADNA, low complement	Mild respiratory symptoms and low-grade arthritis flare.
5	37	F	10	*11:01, *15:01	Neuropsychiatric SLE (epilepsy, headache), arthritis, serositis, leukopenia, positive ADNA.	Mild respiratory symptoms.
6	50	F	37	*01:01, *15:01	Nephritis, aseptic peritonitis, arthritis, positive ADNA and aPL, low complement	Mild respiratory symptoms.
7	44	F	24	*13:01, *16:01	Serositis, leukopenia, thrombocytopenia, interstitial lung disease, positive ADNA and aRNP, low complement	Mild respiratory symptoms.
8	28	F	7	*01:01, *15:01	Arthritis, photosensitive rash, positive ADNA and anti-Sm, DVT+pulmonary embolism	Mild respiratory symptoms.
9	27	M	2	*01:03, *13:02	Fever, photosensitive rash, oral ulcers, arthritis, nephritis, myocarditis, positive ADNA and aRNP, low complement	Mild systemic symptoms

Appendix 4: candidate antigens for antigen-specific T cell studies

Candidate antigen	References	Clinical evidence		Experimental evidence			Reasons for exclusion
		Prevalence in SLE cohort	Prevalence in the general population	Peptide sequences available	T-cell immuno-genicity	HLA binding	
Autoantigens							
Smith antigen	(Zhao et al., 2019b)	Ab: 21%	NA	Yes	Yes	Yes	Low prevalence in the study cohort
U1-RNP	(Kattah et al., 2015)	Ab: 18%	NA	Yes	Yes	No	Low prevalence in the study cohort. Non SLE-specific antigen. Poor results with patients with SLE
β2-GPI	(Benagiano et al., 2019, de Moerloose et al., 2017, Salem et al., 2018, Salem et al., 2015)	Ab: 19%	NA	Yes	Yes	No	Low prevalence in the study cohort. Limited number of patients reported in the literature, no reported HLA compatibility with the present study.
Peptide surrogate for DNA	(Putterman & Diamond, 1998)	Ab: 77%	NA	Yes	No	No	Too limited evidence from murine studies.
Histone H1	(Bruns et al., 2000, Bruschi, Moroni et al., 2021, Lu et al., 1999)	Ab: 77%*	NA	No	No	No	Non-nucleosome component. Too limited evidence.
Histone H2			NA	Yes	Yes	No	
Histone H3			NA	Yes	Yes	No	NA
Histone H4			NA	Yes	Yes	No	
Allergens							
Jug r2 (walnut)	(Archila et al., 2015, McWilliam, Koplin et al., 2015)	<1%	2%	Yes	Yes	No	Low prevalence in the study cohort and in the general population.
Poa p1, Lol p1, Lol p5a, Poa p5a, Phl p1, Phl p5a (Graminaceae)	(Archila et al., 2014, Olivieri, Verlato et al., 2002) (Macaubas et al., 2006)	5%	12%	Yes	Yes	No	Low prevalence in the study cohort. Heterogeneity of antigens.
Der p1 (dust mites)	(Boonpiyathad, Sokolowska et al., 2019, Olivieri et al., 2002)	5%	15%	Yes	Yes	Yes	Low prevalence in the study cohort. Reference publication after preliminary analysis.
Beta-lactams	(Li et al., 2020)	15%	2%	Yes	Yes	Yes	NA
Antigens from infectious agents							
Influenza A PB1 or Haemagglutinin	(Uchtenhagen, Rims et al., 2016)	ND	ND	Yes	Yes	Yes	Heterogeneity of antigens. Limited evidence of a pathogenic role in SLE.
CMV	(Draborg et al., 2018, Pordeus et al., 2008)	ND	77%	ND	ND	ND	Limited evidence of a pathogenic role in SLE.
EBNA 1 (EBV)	(Munz et al., 2000)	ND	83%	No	No	No	NA
EBNA 2 (EBV)	(Leogrande & Jirillo, 1993, Long et al., 2013, Meckiff et al., 2019, Pordeus et al., 2008)			Yes	Yes	Yes	NA

* Assuming a substantial overlap among anti-nucleosome/histone antibodies and anti-DNA antibodies (Bruns et al., 2000). Abbreviations. Ab: antibodies; CMV: Cytomegalovirus; EBV: Epstein-Barr virus

Appendix 5: extended shortlist of peptides for T cell studies

Antigen	HLA restriction	Peptide sequence (aminoacid number)	IEDB adjusted rank	Reference
Autoantigens				
Histone H4	DRB1*03:01	GLIYEETRGVLKVFL (49-63)	17.00	(Lu et al., 1999)
		VVYALKRQGRTLYGFG (87-102)	1.50	ND
Histone H3	DRB1*11:01	LPFQRLMREIAQD (66-78)	1.47	ND
Allergens				
Penicilloylated albumin	DRB1*03:01 DRB1*11:01	PELLFFAK*RYKAAFT	26.00 2.00	(Azoury et al., 2018)
Antigens from infectious agents				
EBNA 2	DRB1*03:01	PAQPPPGVINDQQLHHLPSG (301-320)	19.00	(Long et al., 2013, Meckiff et al., 2019)
		PPGVINDQQLHHLP (305-317)	4.37	ND
		TYHLIVDTDSLGNP (13-26)	6.14	ND
	DRB1*11:01	GPLASAMRMLWMANY (125-139)	19.00	ND
		MPTFYLALHGGQTY (1-14)	10.66	ND
EBNA 1	DRB1*03:01	EVLKDAIKDLVMTK (573-586)	9.48	ND
		AEVLKDAIKDLVMTK (572-586)	12.00	ND
	DRB1*11:01	HIFAEVLKDAIKDL (569-582)	22.61	ND

Appendix 6: clinical features of patients included in T-cell studies

Subject ID	Sex, Age (y)	HLA-DRB1	Diagnosis	Disease duration (y)	Disease activity		HCQ	PDN dose (mg/day)	Immunosupp.
					SLEDAI-2K	LLDAS			
#1*	F, 32	3	SLE	12	4	1	Yes	0	MMF
#1b	F, 30	3	SLE	10	10	0	Yes	0	AZA
#2	F, 25	11	SLE	7	2	1	Yes	3.75	MMF
#3*	F, 47	11	SLE	11	0	1	Yes	5	MMF
#3b	F, 45	11	SLE	10	8	0	Yes	5	AZA
#4	F, 25	11	SLE	8	30	0	No	37.5	IVIG
#5	F, 56	3	SLE	28	2	1	Yes	5	MMF
#6	F, 36	11	SLE	15	12	0	Yes	5	MMF
#7	F, 51	3 and 11	SLE	36	1	1	No	5	None
#8	F, 34	3	SLE	16	2	0	Yes	0	None
#9	F, 59	11	SLE	12	4	1	Yes	2.5	None
#10*	F, 35	11	SLE	7	0	1	Yes	5	MMF
#10b	F, 34	11	SLE	6	9	0	Yes	6.25	AZA
#11	M, 53	11	SLE	19	2	1	Yes	0	None
#12	F, 43	3	SLE	17	0	1	Yes	0	MMF
#13*	F, 34	11	SLE	12	4	1	Yes	5	MMF
#13b	F, 33	11	SLE	11	13	0	Yes	5	None
#14	F, 48	3	SLE	8	0	1	Yes	0	None
#15	F, 35	11	SLE	25	2	1	Yes	0	None
#16	F, 38	11	SLE	22	6	0	Yes	0	None
#17	F, 57	3	SLE	22	8	0	Yes	2.5	MMF
#18	F, 61	11	SLE	32	0	1	Yes	0	None
#19	F, 58	11	SLE	34	2	1	Yes	0	None
#20	M, 45	11	SLE	24	0	1	Yes	0	AZA
#21	F, 23	11	SLE	7	2	1	Yes	0	None
#22	F, 38	3	SLE	19	2	1	Yes	0	None
#23	F, 25	11	SLE	9	9	0	Yes	5	MTX
#24	F, 35	3	SLE	22	2	1	No	2.5	None
#25*	F, 26	3	SLE	5	4	1	Yes	0	MMF
#25b	F, 24	3	SLE	4	16	0	Yes	5	None
#26	M, 42	3 and 11	SLE	8	4	0	Yes	2.5	None
#27	F, 52	11	SLE	19	4	1	Yes	2.5	MTX
#28	F, 39	3	SLE	20	8	0	No	0	AZA
#29	F, 51	11	SLE	26	4	1	Yes	1.25	MMF
#30	F, 44	3	SLE	11	2	1	Yes	0	None
#31	M, 31	3	SLE	1	6	0	Yes	0	MMF
#32	F, 64	3	SLE	36	2	1	Yes	2.5	MTX

Continued at next page

Continues from previous page

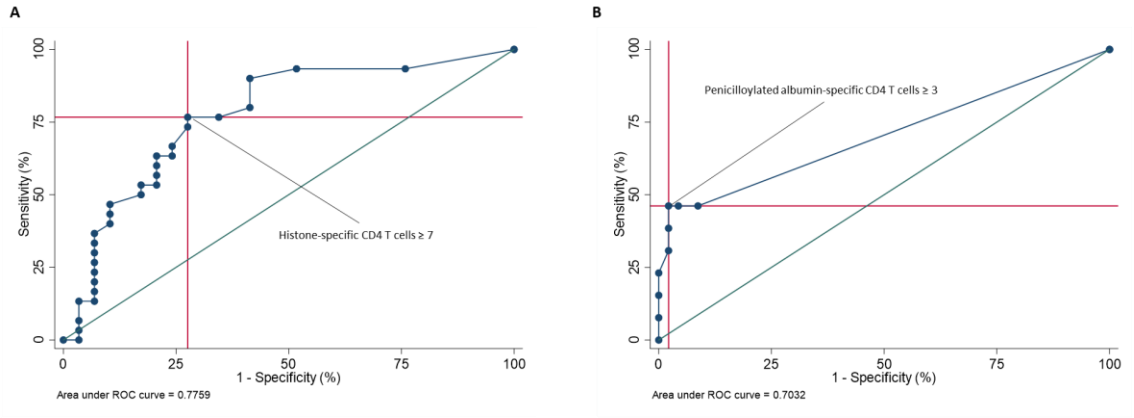
Subject ID	Sex, Age (y)	HLA-DRB1	Diagnosis	Disease duration (y)	Disease activity		HCQ	PDN dose (mg/day)	Immunosupp.
					ITAS	Clinical impression			
#33	F, 36	11	TAK	12	3	Smouldering	No	5	sirolimus
#34	F, 27	3	TAK	5	0	Remission	No	5	MTX, Infliximab
#35	F, 65	3	TAK	17	1	Active	No	0	MTX
#36	F, 46	11	TAK	28	0	Remission	No	5	MTX, Tocilizumab
#37	F, 39	11	TAK	12	2	Remission	No	10	Abatacept
#38	F, 35	3	TAK	6	0	Remission	No	0	AZA
#39	F, 47	11	TAK	7	0	Smouldering	No	0	MTX
#40	F, 44	3	TAK	9	0	Remission	No	5	AZA, Infliximab
#41	F, 47	11	TAK	17	0	Remission	No	5	None
#42	M, 22	11	TAK	5	1	Smouldering	No	10	MTX, Infliximab
#43	F, 50	11	TAK	10	1	Remission	No	5	MTX, Infliximab

Subject ID	Sex, Age (y)	HLA-DRB1	Diagnosis
#44	M, 25	11	HC
#45	F, 33	3	HC
#46	F, 59	3	HC
#47	F, 32	11	HC
#48	F, 42	3	HC
#49	F, 51	11	HC
#50	F, 25	3	HC
#51^	F, 25	3 and 11	HC
#52	F, 31	11	HC
#53	F, 48	3	HC

*: re-tested during active disease. ^tested twice

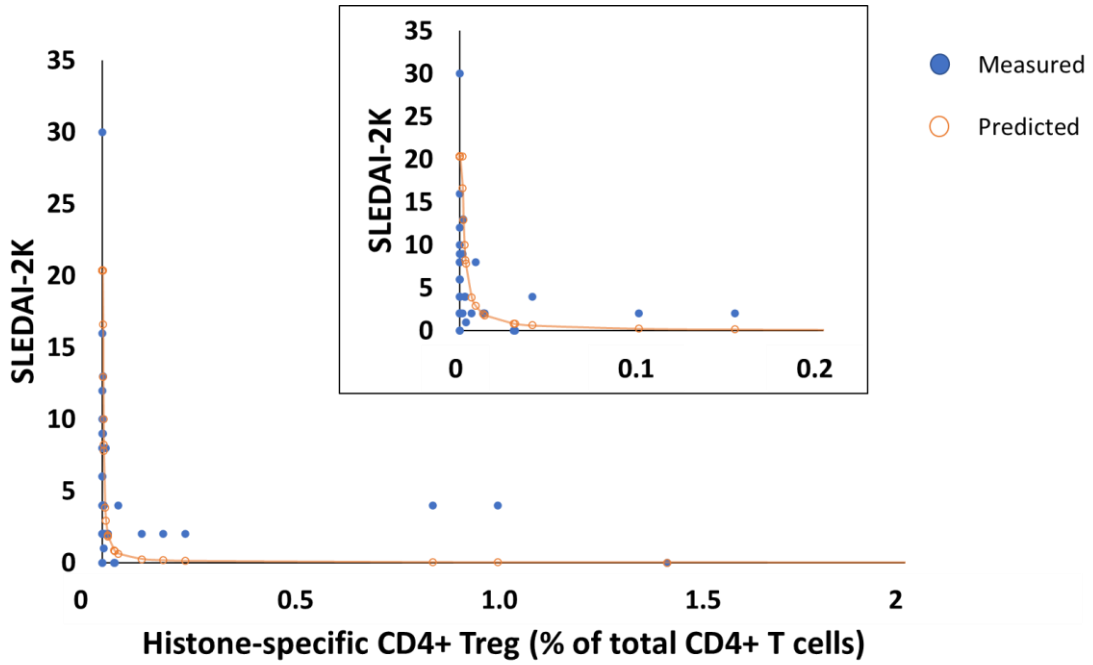
Abbreviations. AZA: azathioprine, HC: healthy control, ITAS: Indian Takayasu Disease Activity Score, IVIG: intravenous immunoglobulins, LLDAS: lupus low disease activity state, MMF: mycophenolate mofetil, MTX: methotrexate, PDN: prednisone, SLE: systemic lupus erythematosus, SLEDAI-2K: SLE disease activity index 2000, TAK: Takayasu's arteritis.

Appendix 7: receiver operating characteristics curves for antigen-specific T cell counts.



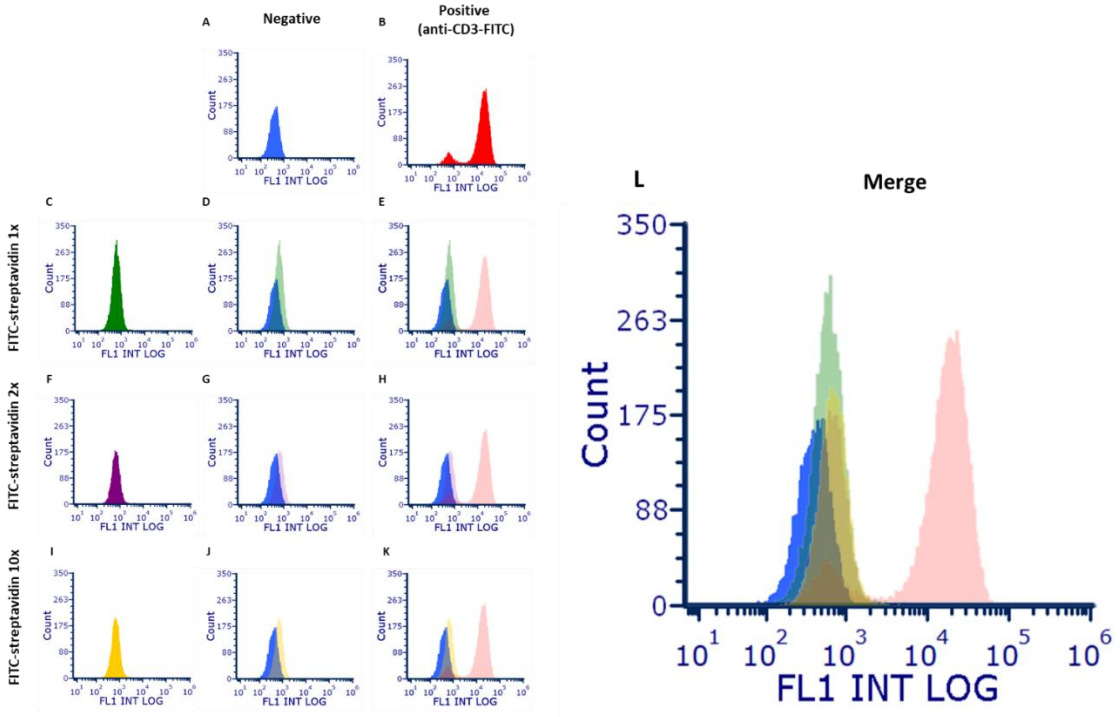
Receiver operating characteristics curves showing the diagnostic performance of total absolute histone-specific (A) and penicilloylated albumin-specific (B) CD4+ T cell counts for identifying subjects with positive antiDNA and allergy to beta-lactams, respectively. The red reference lines highlight sensitivity and 1-specificity values of optimal cut-offs for both continuous variables.

Appendix 8: curve fitting for SLEDAI-2K and histone-specific T-reg CD4+ T cells



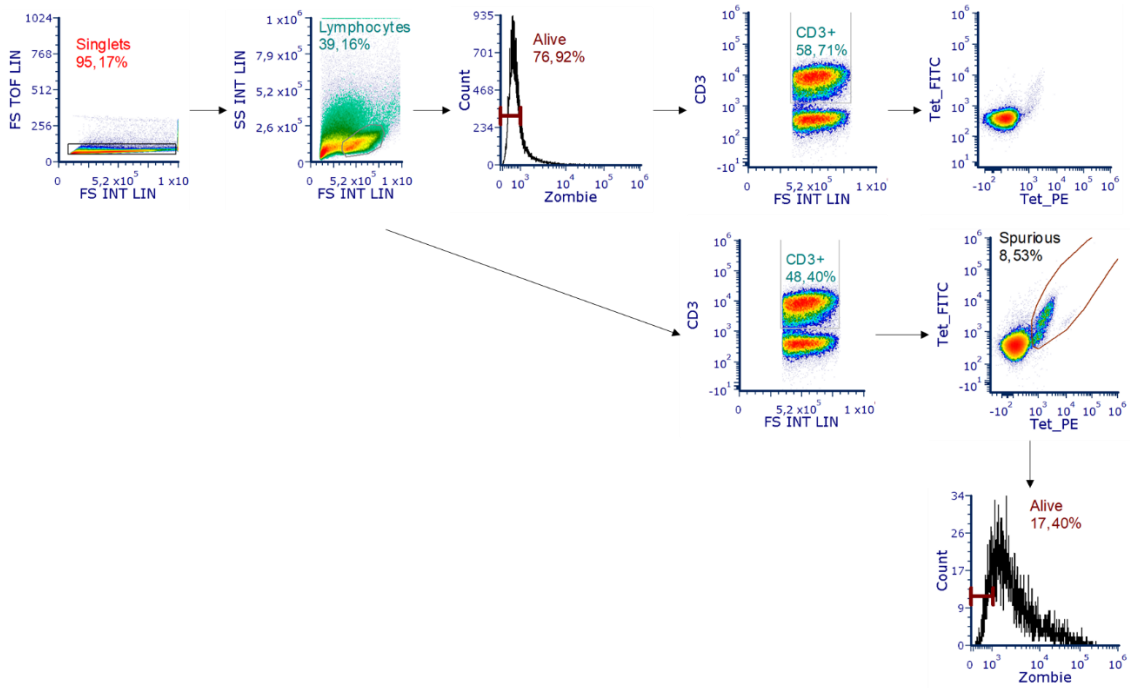
This figure shows the inverse relation between histone-specific CD4+ Treg cells and SLEDAI-2K. Measured SLEDAI-2K / histone specific CD4+ Treg percentages of CD4+ cells are depicted in blue. Orange empty dots and lines show the hyperbolic interpolation curve generated by calculating expected SLEDAI-2K values based on histone-specific CD4+ Treg cell % of total CD4 cells. The internal subpanel shows the curve fit for lower percentages of In particular, the curve had the following equation: $SLEDAI\_2K = \frac{0.25}{(1000 * HTreg\%CD4)}$. For non-null values the curve had a good fit with real SLEDAI-2K values (R^2=5.616 ; p<0.001), whereas it was unable to predict SLEDAI-2K for null histone-specific CD4+ Treg cells.

Appendix 9: FITC-streptavidin staining



Histograms showing the fluorescence intensity of FITC-streptavidin staining on PBMC from a healthy donor in comparison with no staining (Negative, panel A) and control positive staining with an anti-CD3-FITC antibody (Panel B) after gating through singlet events and lymphocyte physical parameters. PBMC were stained with 1x (panel C-E), 2x (panel F-H), 10x (panel I-K) the concentration of FITC-streptavidin employed to generate FITC-MHC tetramers. Panel L shows a merged view of the previous panels. FITC-streptavidin alone did not generate a significant fluorescence compared to the negative (unstained) and positive controls. Data were acquired with a Beckman Coulter Navios Flow Cytometer, elaborated into graphs with FCS Express version 7 and merged with Microsoft PowerPoint 2019.

Appendix 10: exclusion gating for spurious events



This figure depicts the gating strategy employed to exclude spurious FITC/PE co-fluorescent signals from flow cytometry analyses employing MHC-FITC/MHC-PE tetramers. In this representative example PBMC from a healthy subject are shown. Most double positive (“spurious”) cells were dead as confirmed by back-gating.

Appendix 11: kinetics of selected T-helper-related cytokines

Cytokine	Timing of measurement/release (h)	Reference
IL2	0.5-4-14-18-24-30	(Hartmann, Marjanovic et al., 2014, Oh, Chen et al., 2016, Parlesak, Haller et al., 2004)
IL4	6-12-24	(Barata, Ying et al., 1998, de Boer, Fillié et al., 1998, Krouwels, Hol et al., 1998)
IL5	6-12-24	(Barata et al., 1998, Krouwels et al., 1998)
IL6	10-18-24-70	(Hartmann et al., 2014, Parlesak et al., 2004, Stanley & Lacy, 2010)
IL9	12-96	(Liu, Harberts et al., 2014, Louahed, Zhou et al., 2001)
IL10	0.5-24-30	(Oh et al., 2016, Parlesak et al., 2004)
IL13	20-40-48	(de Boer et al., 1998, Ohshima, Yang et al., 1999)
IL17A	4-6-48	(Ferretti, Bonneau et al., 2003, He, Lang et al., 2013, Naji, Smith et al., 2014)
IL17F	4-24-36-72	(Burns, Maroof et al., 2020, Duhen, Geiger et al., 2009, Garcia-Arellano, Hernandez-Palma et al., 2018, Naji et al., 2014, Saini, Ramesh et al., 2013)
IL22	18-36-72	(Duhen et al., 2009, Perriard, Mathias et al., 2015, Zheng, Danilenko et al., 2007)
IFN-γ	0.5-6-24-24-48-70	(Krouwels et al., 1998, Oh et al., 2016, Ohshima et al., 1999, Parlesak et al., 2004, Stanley & Lacy, 2010)
TNF	0.5-10-18	(Hartmann et al., 2014, Oh et al., 2016, Parlesak et al., 2004, Stanley & Lacy, 2010)