

# Abstracts

## Short talk presentations

### **Heightened extrafollicular B cell responses influenced by oxysterol and sex hormone availability contribute to female-biased autoimmunity**

Nina de Gruijter<sup>1,2</sup>, Diana Matei<sup>1,2</sup>, Hannah Peckham<sup>1,2</sup>, Beth Jebson<sup>1,3</sup>, Anna Radsizewska<sup>1,2</sup>, Coziana Ciurtin<sup>1,2</sup>, Elizabeth C. Rosser<sup>1,2</sup>

<sup>1</sup> Centre for Adolescent Rheumatology Versus Arthritis at UCL UCLH and GOSH, London, UK

<sup>2</sup> Centre for Rheumatology Research, Division of Medicine, University College London, London, UK

<sup>3</sup> UCL Great Ormond Street Institute for Child Health, UK

Emerging evidence suggests that inappropriate extrafollicular B cell activation plays a critical role in the generation of autoantibody-producing B cells in systemic lupus erythematosus (SLE). Previous studies have demonstrated that interaction of the oxidised cholesterol receptor GPR183 with its main ligand -alpha-25-dihydroxycholesterol is critical in regulating the early differentiation of plasmablasts and B cell positioning into extrafollicular sites. Here, using the parent into F1 model of experimental lupus, we demonstrate that blocking GPR183-driven responses reduces nephritis severity by reducing early, extrafollicular plasmablast differentiation. However, mimicking the strong female-bias observed in human lupus, the efficacy of GPR183-antagonism in suppressing lupus pathogenesis and plasmablast differentiation was only observed in female mice and could not be recapitulated in male animals. Interrogation of the mechanisms underlying this dichotomy revealed that this was due to underlying sex differences in the magnitude of extrafollicular B cell activation post-lupus induction. Notably, modulating sex-hormone receptor signalling or changing oxysterol availability through a high fat-diet ablated any observed sex differences in lupus severity suggesting a dynamic interplay between these systems. Importantly, these data were recapitulated in humans as there are higher baseline levels of extrafollicularly-derived IgD-CD27- DN B cells in healthy post-pubertal cis-females compared to cis-males and a general dysregulation in these pathways in patients with juvenile-onset SLE compared to controls. These data suggest a dynamic interplay between sex hormones levels, oxysterol metabolism and extrafollicular B cell activation which impacts female biased autoimmune disease risk.

## **Human lymph node stromal cells maintain FOXP3+ regulatory T cells in a HLA-DR dependent manner**

A M O'Byrne, C. Grasso, J.F. Semmelink, E.M. Remmerswaal, J.W. Bolt, R.E. Mebius\*,  
L.G.M. van Baarsen\*

Amsterdam University Medical School, Netherlands

Animal studies show that lymph node stromal cells (LNSCs) are key players in peripheral tolerance through self-antigen presentation to T cells thereby controlling auto reactivity. Thus, dysfunctional LNSCs may play a role in the breaking of tolerance observed in autoimmune diseases like rheumatoid arthritis (RA). We hypothesize that human LNSCs from individuals without autoimmunity can express HLA-DR and maintain homeostasis of FOXP3+ regulatory T cells (Treg), a process potentially perturbed in RA LNSCs.

Lymph node needle biopsies were collected from healthy volunteers, autoantibody positive RA-risk individuals and RA patients. Flow cytometry and qPCR techniques were used to determine the expression of HLA-DR directly ex vivo and on in vitro cultured LNSCs. To investigate the ability of LNSCs to maintain Tregs we employed an in vitro coculture system of autologous LNSCs and CD4 T cells. Subsequently flow cytometry analysis was used to determine the presence of FOXP3+ Tregs. The presence of lymphocytes induced HLA-DR expression on cultured LNSCs. Our results show that the presence of LNSCs is crucial for maintaining FOXP3+ Tregs. We used blocking antibodies for both HLA-DR and interleukin 2 (IL-2) to show that both were required for Treg maintenance. In the context of autoimmunity we showed that LNSCs from RA patients directly analyzed ex vivo have a significantly reduced frequency of HLA-DR+ LNSCs compared to control and RA-risk individuals.

## **DNASE1L3 at the forefront of intestinal mucosal immunity**

Lucia Montorsi<sup>1</sup>, Michael J. Pitcher<sup>1</sup>, Alicia Demonti<sup>1,2</sup>, Pawan Dhami<sup>3</sup>, Richard J. Ellis<sup>4</sup>, Cynthia Bishop<sup>4</sup>, Mats Bemark<sup>5</sup>, Jo Spencer<sup>1</sup>

<sup>1</sup> School of Immunology and Microbial Sciences, King's College London; London, UK

<sup>2</sup> École Normale Supérieure de Lyon, Claude Bernard Lyon 1 University; Lyon, France

<sup>3</sup> Genomics Research Platform and Single Cell Laboratory at Guy's and St Thomas' NHS Foundation Trust; London, UK

<sup>4</sup> Flow Cytometry and Immune Monitoring Platform at Guy's and St Thomas' NHS Foundation Trust; London, UK

<sup>5</sup> Department of Microbiology and Immunology, University of Gothenburg; Gothenburg, Sweden

Gut-Associated Lymphoid Tissue (GALT) is composed of lymphoid follicles which lie directly underneath a specialized form of epithelium called follicle associated epithelium (FAE). The FAE is known to sample antigen from the gut lumen and transport them into the sub-epithelial dome (SED) where they are internalized and processed by specialized dendritic cells (DC). GALT follicles act as sites of local priming and expansion of B cell through germinal-centre and extrafollicular proliferation, while also having to maintain tolerance to commensal microbiota and dietary antigens, although the specific mechanisms underlying the maintenance of this delicate balance are poorly understood.

To comprehensively characterize these structures, we performed a spatial transcriptomic analysis on human GALT follicles using Visium 10X technology. Thanks to this analysis, we identified DNASE1L3 transcript to be specifically expressed in the sub-epithelial region of human GALT alongside the complement component C1q. Using a combined method to co-detect protein and mRNAs targets simultaneously in the Imaging Mass Cytometry setting, we showed that DNASE1L3 is localized in CD68+CD11c+ DCs that also express bactericidal enzymes Lysozyme and NOX2. Surprisingly, we observed that DNASE1L3 in human is not expressed by germinal centres macrophages specialized in uptaking apoptotic debris, while this is true in mouse models. Finally, we show that internalized bacteria are localized in the SED in proximity to DNASE1L3+ cells but are absent from the rest of the follicle. In conclusion, we identified for the first time a population of DCs expressing DNASE1L3 in the SED of human GALT and suggest that DNASE1L3 might be involved in managing of bacterial DNA rather than eukaryotic DNA derived from apoptotic debris, as previously hypothesized. This might be clinically relevant in the context of Lupus disease where DNASE1L3 is often functionally inactivated, suggesting bacterial DNA might play a role in the generation of pathogenic anti-DNA antibody response.

## **Checkpoint inhibitor-associated inflammatory arthritis is comprised of multiple clinical endotypes characterized by distinct transcriptional programs**

Gary Reynolds<sup>1,2</sup>, Mazen Nasrallah<sup>1</sup>, Neal Smith<sup>1</sup>, Steven Blum<sup>1</sup>, Molly Thomas<sup>1</sup>, Daniel Zlotoff<sup>1</sup>, Kerry Reynolds<sup>1</sup>, Andrew Luster<sup>1</sup>, Alexandra-Chloé Villani<sup>1</sup>

<sup>1</sup> Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital, Boston, MA, USA

<sup>2</sup> Newcastle University, UK

### Background:

ICI-associated inflammatory arthritis (ICI-IA) affects around 5% of patients on ICI therapy. Clinical manifestations mimic those of immune-mediated rheumatic diseases (IMRDs) such as rheumatoid arthritis. However, in contrast to IMRDs which develop over decades, the perturbation that provokes ICI-IA is clearly defined so provides a unique opportunity to explore triggers of autoimmunity.

### Methods:

Comprehensive clinical and demographic data were collected for patients with malignancy, treated with ICI, and subsequently diagnosed with inflammatory arthritis (n=41). Peripheral blood and synovial fluid were collected and profiled. Presentations of ICI-IA included mono/oligoarthritis (n=9 profiled) and polyarthritis (n=7 profiled). Samples were analysed by paired single cell RNA sequencing, surface proteome and T cell receptor (TCR) sequencing.

### Results:

Patients presenting with monoarthritis were ANA negative (0/6) with higher abundance of DC1 and DC2 (Dirichlet regression,  $p=1.1 \times 10^{-3}$  and  $p=8.1 \times 10^{-5}$ ). Patients presenting with polyarthritis were significantly more likely to be antinuclear antibody positive (15/26, Fisher's test,  $p=0.04$ ) with expansions of macrophages expressing interferon response genes (IFI44L, ISG15). Comparing datasets from rheumatoid and psoriatic arthritis, we identified lymphocytes specific to ICI-IA resembling those from melanoma tumour.

### Conclusions:

These data demonstrate that ICI-IA is clinically and immunologically heterogeneous. Furthermore, it contains cell states that are unique to ICI-IA and distinct from those in common IMRDs. This suggests that ICI-IA does not represent an unmasking of latent autoimmunity and that, despite a well-defined precipitant, complex individual and environmental factors combine to produce a heterogeneous disease.