leading to the formation of these autoantibodies. At present it is unknown how AMPA are generated and how autoreactive B-cell responses against PTM proteins are induced.

Objectives: To investigate how PTM proteins, more specifically carbamylated proteins, could contribute to a breach of B-cell tolerance.

Methods: Serum reactivity towards five different carbamylated proteins was determined for 160 RA-patients and 40 healthy individuals. Anti-CarP antibody cross-reactivity was studied by inhibition experiments. Mass spectrometry was performed to identify carbamylated self-proteins in human rheumatic and osteoarthritic joint tissue. Mice were immunized with carbamylated- or non-modified foreign (OVA) and self-antigens (mouse Albumin). Sera of immunized mice and monoclonal antibodies were analyzed for antigen reactivity.

Results: We show that anti-CarP antibodies in RA are highly cross-reactive towards multiple carbamylated proteins, including modified self- as well as modified non-self proteins. Immunization with carbamylated foreign proteins (Ca-OVA) induced a strong anti-CarP response against both carbamylated foreign- and self-proteins. Similar to murine serum anti-CarP antibodies, murine monoclonal anti-CarP antibodies were highly specific and cross-reactive to multiple carbamylated (auto)antigens. Although citrulline greatly resembles homocitrulline residues in structure, murine anti-CarP antibodies differ in antigen recognition profile from ACPA as they are able to discriminate between citrullinated and homocitrullinated forms of the same protein. Interestingly, we were able to identify carbamylated-albumin, in RA synovial tissue, indicating that albumin is present in carbamylated form locally in the inflamed joint.

Conclusions: Self-reactive AMPA-responses can be induced by exposure to foreign proteins containing PTM. Our findings show that autoreactive B-cell responses against PTM self proteins can be induced by exposure to PTM foreign proteins and provide new insights on the breach of autoreactive B-cell tolerance by foreign proteins.

Disclosure of Interest: None declared

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SAT0031 DETECTION AND ISOLATION OF ANTIGEN SPECIFIC B CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: The majority of autoimmune diseases, e.g. rheumatoid arthritis (RA), are characterized by autoantibodies that are produced by B cells. We have identified several novel post-translationally modified epitopes in collagen type II (CII), a major cartilage constituent that are autoreactive. In mouse models, autoantibodies that bind to these epitopes can either induce or protect against inflammatory arthritis. However, very little is known about the frequencies or phenotype the B cells that produce these autoantibodies in humans.

Objectives: The aim of this project is to study the B cells that produce autoantibodies reactive to CII-epitopes in patients with RA.

Methods: Clinical data and peripheral blood were collected from patients with RA (n=100). Titres of CII-reactive serum-autoantibodies were determined by Luminex. The different subsets of B cells that expressed a CII-reactive B cell receptor (BCR) were analysed and isolated in patients with positive (n=10) or negative (n=5) titres for autoantibodies recognizing the CII-epitopes cyc48 (CII-F4-R-Cit) and cyc49 (CII-F4-Cit-R) as well as in healthy controls (n=3) using flow cytometry.

Results: In patients with detectable autoantibodies to cyc48 and cyc49, their titres were 13761±3815 and 5585±1992 (mean ± SEM), respectively. The corresponding titres in the patients negative for these autoantibodies were 122±55 and 234±75. In autoantibody-positive patients, the frequencies of cells expressing a BCR reactive for cyc48 and cyc49 in different subsets were: transitional cells 0.88±0.18% and 1.33±0.33%; naïve cells 0.09±0.0008% and 0.13±0.06% and memory cells 0.31±0.25% and 0.30±0.12%. All frequencies were well above those detected in patients without cyc48/49 titres and in healthy controls. A higher proportion (p=0.03) of the cyc48/49 positive patients were treated with methotrexate compared to cyc48/49 negative patients, no other clinical differences were recorded. CII-reactive single B cells were isolated in order to clone the BCR

Conclusions: In patients with manifest RA there is a relatively high and detectable frequency of transitional B cells that express a joint-specific BCR, which suggests that the deletion of autoreactive B cells in the bone marrow in RA patients is defective. As there is a decrease in the proportion of naïve CII-specific B cells, a substantial amount of the autoreactive cells are deleted in the periphery. However, peripheral B-cell tolerance is incomplete, as CII-specific B cells are enriched in the pool of memory B cells.

Disclosure of Interest: None declared

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SAT0032 EX VIVO ANALYSIS OF AUTOANTIGEN-SPECIFIC T CELL RESPONSES USING A MULTI HLA-CLASS II TETRAMER APPROACH

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Background: HLA class II tetramers allow the direct *ex vivo* enumeration and phenotypic characterization of antigen-specific T cells and have proven a useful tool in different settings, e.g. allergy and vaccination.

In rheumatoid arthritis (RA), many candidate auto-antigens have been characterized and are believed to play a role in the disease.

Objectives: Based on flow cytometry we developed a multi HLA-class II tetramer approach that renders it possible to look at numerous specificities simultaneously and is sufficiently sensitive at the same time as auto-reactive T cells are likely to be rare. We focused on validated citrullinated T cell epitopes previously studied in RA, namely a-enolase, fibrinogen and CILP.

To test the robustness and sensitivity of our multi-tetramer assay, we performed repeated experiments on PBMCs of HLA-DRB1*04:01-positive healthy controls as well as HLA-DRB1*04:01-positive RA patients.

Results: First, we examined the sensitivity of the panel by assessing PBMC from healthy donors and we could detect low frequencies of auto-reactive T cells in these samples (1–10 per million CD4), mostly displaying a naïve phenotype, which was in sharp contrast to influenza-specific T cells in the same donors which were 10–20 fold higher in numbers and of memory phenotype. Next, we tested the robustness of the panel by running technical repeats of all healthy donor samples, which yielded similar frequencies. Thereafter, we focused on RA patient PBMC obtained from repeated blood draws (2–4 weeks apart). Also in these samples, frequencies ranged between 1–10 tetramer-positive cells per million CD4+ T cells, i.e. similar as in healthy controls. Moreover, not all T cell specificities were present in all patients. Still, we found the frequencies to be stable in the repeated blood draws in approximately half of the individuals, implicating that frequencies close to 1 cell per million CD4+ T cells is borderline of what we can stably detect. Importantly, the patient samples utilized were not taken from time points of active disease, where we hypothesize frequencies to be elevated. This may be the case also in synovial fluid and as a proof of principle, we assessed synovial fluid samples of HLA-DRB1*04:01+ RA patients and were able to detect auto-reactive T cells at frequencies comparable to the ones found in peripheral blood.

Conclusions: In conclusion, we developed a sensitive tetramer panel allowing the simultaneous enumeration and phenotypic characterization of antigen-specific T cells in *ex vivo* samples from RA patients that can be used for monitoring and in-depth studying of these cells during the course of disease and treatment in individual patients.

Disclosure of Interest: None declared

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SAT0033 SMOKING CONTRIBUTES TO EXHAUSTED STATE OF CD4+ T CELLS IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) has recently been linked to an exhausted state of CD4+ T cells in peripheral blood of patients [1]. Exhaustion of CD4+ T cells limits their proliferation and increases cell death. In CD8+ T cells smoking counteract exhaustion, which may lead to increased cytotoxic activity exemplified by targeting of cells with high expression of the anti-apoptotic protein survivin [2]. Exhaustion of CD4+ T cells coincides with expression of interferon (IFN) response genes [3], referred to as the IFN signature. The development of the IFN signature has been suggested to predate RA [4].

Objectives: We investigated how smoking affect the CD4+ T cell population in peripheral blood of RA patients with focus on the exhaustion marker programmed cell death-1 (PD-1).

Methods: Blood samples were collected from RA patients and healthy women with different smoking status and analysed for PD-1 and survivin expression using flow cytometry and qPCR. Sorted Th17 cells from peripheral blood were analysed for expression of 18 genes up regulated during exhaustion [3], herein referred to as the exhaustion set, and serum levels of survivin were assessed by ELISA. Peripheral blood CD4+ cells were analysed for their expression of seven IFN response genes [4]. The role of survivin in the formation of exhausted CD4+ T cells was studied in collagen-induced arthritis (CIA), where mice were treated with nicotine or vaccinated with survivin peptides.

Results: High frequency of exhausted PD-1+CD4+ cells was found in smoking RA patients. The numbers of PD-1+CD4+ cells correlated inversely with the PD-1 expression by cytotoxic CD8+CD107+ cells (r=-0.62, p=0.01). Additionally, the frequency of PD-1+CD4+ cells increased with reduction of the CD4+ population (r=-0.71, p=0.002). The IFN signature was found exclusively among smoking RA patients. The patients with the IFN signature all had CD4+ cells with low survivin

production. Th17 cells from RA patients with high serum survivin were enriched in genes of the exhaustion set. CD4⁺ cells with high survivin expression were negative for PD-1, while PD-1^{hi} cells had low expression of survivin. In CIA mice the survivin^{hi}PD-1⁺ CD4⁺ cells were reduced by nicotine treatment ($p=0.03$) or survivin vaccination ($p=0.009$).

Conclusions: Smoking associates with exhaustion of CD4⁺ T cells in RA by increasing the frequency of PD-1⁺CD4⁺ cells and supporting the IFN signature. Balancing T cell exhaustion and preventing the IFN signature are potential future treatment strategies for RA.

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SAT0034 ANALYSIS OF THE T-CELL SUBSET COMPOSITION IN ANKYLOSING SPONDYLITIS PATIENTS WITH LONG-STANDING ANTI-TNF THERAPY

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Background: Ankylosing spondylitis (AS) is a chronic, progressive, immune-mediated inflammatory disease, driven primarily by Th1 and Th17 cells. Anti-TNF therapies are successfully used in AS to achieve and maintain remission. However, their influence on the composition of the T-cell repertoire is not clear, and, in particular, the few published studies involve mostly patients with anti-TNF treatment of short duration.

Objectives: We aimed to characterize the changes in several T cell subsets after long-term anti-TNF treatment in AS patients.

Methods: Twenty-two AS patients on long-term anti-TNF therapy were evaluated (15 anti-TNF-responders and 7 non-responders). A wide range of cell subtypes were analysed with flow cytometry and compared with therapy-naïve and short-term data.

Results: Key findings include decreased proportions of naïve CD4 and CD8 cells, increased frequencies of Th1 and Th17 cells and higher Th1/Th2 and Th17/Treg ratios in the long-term anti-TNF-treated patients (responders, non-responders and total), which was found to be significant not only when compared with healthy controls, but also with therapy-naïve and short-term anti-TNF-treated AS patients. We have found several alterations within the various activated T-cell subsets – increase in CD4HLADR cells in responders, in CD8HLADR cells in the whole AS group and in responders, and in CD4CD25 cells in responders, and decrease in CD4CD69 cell percentages in long-term treated patients – becoming evident only after long-term anti-TNF-therapy.

Conclusions: This study provides a comprehensive assessment of the impact of anti-TNF therapy on the T cell repertoire in AS, and indicate that these therapies induce profound changes within T-cells. Changes in T cell phenotype seem to develop progressively during therapy, even in inactive disease, and reflect an ongoing effector T-cell differentiation and activation, and a normalization of Treg development.

Disclosure of Interest: None declared

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SAT0035 INHIBITION OF PROTEIN KINASE C THETA BY THE SELECTIVE INHIBITOR CC-90005 INDUCES T CELL ANERGY

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Background: Protein Kinase C theta (PKC-θ), a member of the PKC family of serine/threonine kinases, is essential in T cell receptor (TCR) signaling and T cell activation [1]. Inhibition of PKC-θ activity may provide new therapeutic options for autoimmune diseases with a T effector cell dependent pathology. CC-90005 is a highly selective small molecule inhibitor of PKC-θ.

Objectives: Evaluate the impact of specific PKC-θ inhibition on T-cell activation and anergy using the highly selective inhibitor CC-90005.

Methods: Human peripheral blood mononuclear cell (PBMC) or isolated T cell cultures were pre-treated with CC-90005 (0.1–10 μM) and stimulated for various times up to 24 h with anti-CD3 and anti-CD28 activation of the TCR, followed by a washout incubation period (1 h–24 h). Cells were then re-stimulated in the presence or absence of CC-90005 and cultured a further 24–96 h. T cell activation was assessed by CD25 and CD69 cell surface expression, thymidine incorporation, and cytokine production.

Results: Upon TCR stimulation, CC-90005 significantly inhibited CD25 and CD69 expression and T cell proliferation in PBMC and isolated T cells. Similarly, CC-90005 inhibited the production of Th1, Th2, Th17 and other pro-inflammatory cytokines. Furthermore, the inhibitory effects of CC-90005 on T cell activation persisted after washout in stimulated T cells, indicating an induction of an anergic

cell state. CC-90005 anergy induction was shown to require only short initial exposures of as little as 2 h and to maintain anergy for as long as 48–96 h post CC-90005 washout. However, T cells pre-treated with the inhibitor in the absence of concomitant TCR stimulation were able to respond to stimulus after as little as 1 h washout, indicating a rapid recovery of PKC-θ activity and T-cell function. CC-90005 upregulated an anergy-related E3 ubiquitin ligase, *Gene related to anergy in lymphocytes (GRAIL)* in T cells. Elevated expression level of GRAIL was maintained in unresponsive T cells after washout, suggesting that GRAIL was a possible downstream mediator of CC-90005 effect on T cell anergy.

Conclusions: CC-90005, a specific inhibitor of PKC-θ, significantly inhibits TCR-mediated T cell activation, proliferation, and cytokine production. Moreover, inhibition of these T-cell functions persists after drug withdrawal and restimulation of T-cells, but only if the primary T cell activation event occurs in the presence of CC-90005. Thus, CC-90005 induces a functional unresponsiveness anergic state in T cells if present during TCR activation, which may have long-term therapeutic benefit in the treatment of T-cell mediated autoimmune and allergic inflammatory conditions.

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Disclosure of Interest: None declared

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SAT0036 IL-17+CD8+ T CELLS ENRICHED IN THE SYNOVIAL FLUID OF PSA/SPA PATIENTS EXHIBIT A PRO-INFLAMMATORY CYTOKINE PHENOTYPE

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Background: Seronegative spondylarthropathies (SpA) share common clinical and immunological characteristics, including a common association with MHC class I, suggesting a role for CD8+ T cells. In contrast, rheumatoid arthritis (RA) is associated with MHC class II. We previously showed that IL-17+CD8+ T cells are increased in the synovial fluid (SF) of patients with psoriatic arthritis (PsA) but not RA, compared to peripheral blood (PB). This study extends this analysis to other SpA types, and phenotypes the SF IL-17+CD8+ T cells to further elucidate their potential pathogenic role.

Methods: Paired PB and SF were collected from patients with RA, PsA and other SpA types (ankylosing spondylitis (AS), reactive arthritis (ReA), enteropathic arthritis (EA) and peripheral SpA (p-SpA)) from Guy's Hospital Rheumatology clinic with informed consent. PB from healthy controls (HC) was collected at King's College London. PBMC and SFMC were isolated and stimulated *ex vivo* with PMA/ionomycin in the presence of golgistop before analysis of surface marker and cytokine expression by flow cytometry.

Results: The percentage of IL-17A+CD8+ T (Tc17) cells was increased in the SF of PsA (median 0.9%, $p=0.0012$, $n=13$) as well as other SpA patients (0.34%, $p=0.0009$, $n=14$), but not RA patients (0.07%, $p=0.3$, $n=7$) compared to matched PB (median 0.10%, no significant differences between disease and control PB). The percentage of SF IL-17A+CD4+ T (Th17) cells was increased in patients with SpA (2.14%, $p=0.004$, $n=14$), RA (2.31%, $p=0.016$, $n=7$) and to a lesser extent PsA (0.97%, $p=0.057$, $n=13$), compared to PB (median 0.61%, no significant differences between disease and control PB).

Phenotypically, most SF IL-17A+CD8+ T cells expressed CCR6 (median 88%, $n=7$) and CD161 (75%, $n=8$), known markers of IL-17+CD4+ T cells. A high percentage also expressed CD103 (69%, $n=9$), which was not observed in the synovial IL-17A+CD4+ T cells (2%, $n=10$). A high frequency of IL-17A+CD8+ T cells co-expressed a range of pro-inflammatory cytokines including IFN-γ (72%, $n=12$), GM-CSF (58%, $n=7$), TNF-α (48%, $n=12$) and to a lesser extent IL-21 (20%, $n=6$) and IL-22 (5%, $n=6$). IL-10 was usually co-expressed at very low levels (1%, $n=7$), although 2 samples showed co-expression of IL-10 (33%, $n=2$). CCR6, CD161, GM-CSF and IL-21 were not significantly co-expressed by either IL-17A negative CD8+ or IFNγ+CD8+ (Tc1) T cells from the SF, indicating this may be a co-expression profile specific to IL-17 expressing T cells.

Conclusions: These findings confirm the presence of an IL-17A+CD8+ T cell subset in the SF of PsA patients and extend this to other SpA types. The expression of the integrin CD103 by these cells indicates they may represent a tissue resident memory (Trm) population in the synovial joint. In addition to IL-17A, the co-expression of several pro-inflammatory cytokines combined with low expression of IL-10, suggests a pro-inflammatory role for these cells.

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