A6.3 IMMUNISATION WITH RECOMBINANT AUTOCITRULLINATED *PORPHYROMONAS GINGIVALIS* PEPTIDYLARGININE DEIMINASE INDUCES AUTOIMMUNITY TO ENOLASE AND ARTHRITIS IN DBA/1 MICE

doi:10.1136/annrheumdis-2013-203220.3

Anne-Marie Quirke, Dany Perocheau, Anna Montgomery, Patrick J Venables. *Kennedy Institute of Rheumatology, Oxford University, London, UK*

Background and Objectives Rheumatoid arthritis (RA) is characterised by the presence of anti-citrullinated peptide antibodies (ACPA) years before disease onset. Increasing molecular and epidemiological evidence has linked periodontitis (PD) to RA. *Porphyromonas gingivalis* is unique amongst periodontal pathogens in possessing a citrullinating enzyme, peptidylarginine deiminase (PPAD) with the potential to generate citrullinated antigens driving the autoimmune response in RA. We have examined the immune response to several peptides/proteins of significance to RA in DBA/1 mice immunised with recombinant PPAD.

Materials and Methods Twelve week old DBA/1 mice were immunised with one of two emulsions: 1) recombinant PPAD in complete Freund's adjuvant (CFA) or 2) an inactive PPAD mutant (C351A) in CFA. Clinical score and paw swelling of mice (indicative of arthritis) recorded for ten days post onset. Antibody responses to PPAD and C351A, and a number of immunodominant ACPA target peptides: anti-citrullinated a-enolase peptide-1 (CEP1), vimentin (cVim), fibrinogen (cFib) and their uncitrullinated forms (REP-1, vim and fib) were examined in mouse serum using Enzyme-linked Immunosorbant assays (ELISAs). The Mann-Whitney U test was used to calculate p-values for differences between the sera groups for each antigen.

Results By day 30 post immunisation, 20% of mice immunised with PPAD had developed arthritis-like swelling in their paws. There was no significant difference between the antibody response to PPAD and the antibody response to C351A in any of the mice tested. There was a significantly raised antibody response ($p < 0.05$) to both CEP1 and REP1 (mean 0.263; OD^{450}) in the mice immunised with PPAD compared to the mice immunised with C351A (CEP1, mean 0.074 (OD^{450}) and REP1 mean 0.150 (OD^{450}). Antibody responses to cFib and Fib were similar in all mice, as were antibody responses to cVim and Vim.

Conclusions The paw swelling and raised immune response to the immunodominant enolase peptide, both citrullinated (CEP1) and uncitrullinated (REP1), in mice immunised with autocitrullinated PPAD shows that PPAD induces arthritis and autoimmunity to enolase. This demonstrates that an active citrullinating PPAD can break tolerance to a major RA autoantigen and provides further molecular evidence linking *P. gingivalis* infection to RA.

A6.4 PERIODONTOPATHOGENS IN RHEUMATOID ARTHRITIS AND PERIODONTAL DISEASE

doi:10.1136/annrheumdis-2013-203220.4

[1]O Laugisch, [2]B Moeller, [3]T Kantyka, [4]PJ Vernables, [2]PM Villiger, [1]A Sculean, [3,5]J Potempa, [1]S Eick. *[1]Department of Periodontology, School of Dental Medicine, Freiburgstrasse 7, 3010 Bern, Switzerland; [2]Department of Rheumatology, Immunology and Allergology, University of Bern, Inselspital, 3010 Bern, Switzerland; [3]Institute of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University of Krakow, Gronostajowa 7, 30–387 Krakow, Poland; [4]Kennedy Institute of Rheumatology, Imperial College London, London, UK; [5]Department of Oral Health and Rehabilitation, University of Louisville, 501 South Preston, Louisville KY 40202-1701, USA*

Background and Objectives A relationship between rheumatoid arthritis (RA) and periodontitis is suggested. Pathogenesis of periodontitis as one of the most common chronic infectious diseases is thought to be host response on subgingival plaque. Among species known to be associated with severe periodontitis, *Porphyromonas gingivalis* plays an important role. Its virulence is most related to cysteine proteases. Moreover, a peptidylarginine deiminase was described to be able to citrullinate microbial and host proteins. The aim of this study was to characterise a group of RA patients for several variables associated with RA and/or periodontitis in comparison with periodontally healthy and periodontitis subjects without RA. In a first part, clinical data of periodontitis and the load of selected periodontopathic species were analysed.

Methods 51 patients with RA, 27 patients with periodontitis and without RA as well as 16 subjects without periodontitis and RA were recruited. Periodontal disease status was determined by using Periodontal Screening index (PSI). Subgingival plaque was analysed semi-quantitatively by PCR followed by a reverse hybridisation (microIdent, Hain Lifescience) for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, *Prevotella intermedia, Tannerella forsythia and Treponema denticola*. In addition, real-time PCR was used to detect very low loads of *P. gingivalis* (detection level 10 bacteria). For statistical analysis Kruskal-Wallis and Mann-Whitney tests were used.

Results Among the 51 RA patients, 45 were characterised positively for periodontitis. 18 (35%) had a severe periodontitis (PSI 4). Analysing the four subgroups (incl. RA with/without periodontitis) showed differences in *A. actinomycetemcomitans* ($p = 0.043$) and *T. denticola* ($p = 0.028$). *P. gingivalis* was detected in 63% of the RA patients and in 49% of the subjects without RA. In all RA patients and in special without periodontitis, *A. actinomycetemcomitans* was found more often ($p = 0.018$ for all, $p = 0.007$ for subjects without periodontitis). In both RA and non-RA subjects, patients with periodontitis had more *T. denticola* in their plaque ($p = 0.026$; $p = 0.042$).

Conclusions *P. gingivalis* induces immune responses which may be of relevance in RA pathogenesis, but other microbes may also play a role in RA associated periodontitis.

This study was supported by a DGP-GABA research grant.

A6.5 SYNOVIAL LYMPHOID STRUCTURES SUPPORT EPSTEIN-BARR VIRUS PERSISTENCE AND AUTOREACTIVE PLASMA CELL INFECTION IN RHEUMATOID ARTHRITIS

doi:10.1136/annrheumdis-2013-203220.5

[1]Cristina Croia, [2]Barbara Serafini, [1]Michele Bombardieri, [1]Stephen Kelly, [1]Frances Humby, [3]Martina Severa, [3]Fabiana Rizzo, [3]Eliana Marina Coccia, [4]Paola Migliorini, [2]Francesca Aloisi, [1]Costantino Pitzalis. *[1]Centre for Experimental Medicine and Rheumatology, William Harvey Research Institute, Queen Mary University of London, UK; [2]Department of Cell Biology and Neuroscience; [3]Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Rome, Italy; [4]Department of Internal Medicine, University of Pisa, Italy*

Objectives Rheumatoid arthritis (RA) is associated with an increased Epstein-Barr virus (EBV) blood DNA load, a robust immune response to EBV and cross-reactive circulating antibodies for viral and self-antigens. However, the role of EBV in RA pathogenesis remains elusive. Here we investigated the relationship between synovial EBV infection, ectopic lymphoid structures (ELS) and immunity to citrullinated self and EBV proteins.

Methods Latent and lytic EBV infection was investigated in 43 RA synovial tissues characterised for presence/absence of ELS and 11 OA samples by RT-PCR, in situ hybridisation and immunohistochemistry/immunofluorescence. Synovial production of anti-citrullinated proteins (ACPA) and anti-citrullinated EBV peptides (VCP1/VCP2) antibodies was investigated in situ or in vivo in the SCID/RA chimeric model.

Results EBV dysregulation was observed exclusively in ELS+ RA, but not OA, synovia as revealed by presence of EBV latent [LMP2A, EBV-encoded small RNA (EBER)] transcripts and EBER+ cells and immunoreactivity for EBV latent (LMP1, LMP2A) and lytic (BFRF1) antigens in ELS-associated B cells and plasma cells, respectively. Importantly, ~20% of synovial plasma cells producing ACPA were

infected with EBV. Furthermore, ELS-containing RA synovia transplanted into SCID mice supported production of ACPA and anti-VCP1/VCP2 antibodies cross-recognised by ACPA. Analysis of CD4+ and CD8+ T-cell localisation and granzyme B expression suggests that EBV persistence in ELS-containing synovia is favoured by exclusion of CD8+ T cells from B-cell follicles and impaired CD8-mediated cytotoxicity.

Conclusions We demonstrated active EBV infection within ELS in the RA synovium that appears to contribute to local growth and differentiation of ACPA-reactive B cells.

A6.6 THE ROLE OF SYNDECAN-4 IN EXPERIMENTAL COLITIS

doi:10.1136/annrheumdis-2013-203220.6

[1]Mareike Fröhling, [2]Dominik Bettenworth, [1]Peter Paruzel, [3]Frank Echtermeyer, [2,4]Andreas Lügering, [1]Thomas Pap, [1]Athanasios Stratis. *[1]Institut of Experimental Musculoskeletal Medicine, University Muenster, 48149 Muenster, Germany; [2]Department of Medicine B, University Muenster, 48149 Muenster, Germany; [3]Department of Anesthesiology and Intensive Care Medicine, Medical University Hannover, 30625 Hannover, Germany; [4]MVZ Portal 10, Muenster, Germany*

Background and Objectives The transmembrane heparan sulphate proteoglycan syndecan-4 (Scd4) has been implicated in cell-matrix adhesion, cell migration, differentiation, proliferation and plays an important role during inflammation in rheumatoid arthritis. Scd4 is a mediator and modulator of inflammatory signals, upon its binding of cytokines Scd4 acts either as a decoy receptor or through the initiation of Scd-dependend signalling, followed by the formation of a Scd4 complex. Cartilage damage is decreased in sdc4-deficient mice, but osteopontin-mediated liver damage is increased. Because of these dual effects we investigate the impact of sdc4 in murine experimental colitis.

Materials and Methods We performed DSS-induced colitis in Scd4$^{-/-}$ and C57BL/6 WT mice. We used weight loss, colon length and histological scoring of colonic modifications to measure the course of colitis. Scd4$^{-/-}$ and WT mice were orally gavaged with 5×10^8 colony-forming units (CFU) of invasive bacterium *Citrobacter rhodentium* (*C. rhodentium*). The changes of body weight and faecal excretion of *C. rhodentium* were monitored for 21 days followed by evaluation of histological changes after infection. The permeability of the colon was examined in vitro by infection of colon samples from Scd4$^{-/-}$ and C57BL/6 WT mice with *C. rhodentium*. The migration behaviour of endothelial human cells (T-84) and scd4-siRNA T-84 knockdown cells was analysed by scratch assay.

Results DSS-treated Scd4$^{-/-}$ mice lost dramatically more body weight compared to the WT mice and the histological damage according to the Dieleman-Score was markedly increased. At day 19 of post infection the clearance of *C. rhodentium* in Scd4$^{-/-}$ mice was markedly prolonged. In vitro infection of colon samples from Scd4$^{-/-}$ mice with *C. rhodentium* revealed a higher permeability for the bacterium compared to WT colon samples. The knockdown of Scd4 in human endothelial T-84 cells leads to delayed cell migration.

Conclusions Like in inflammatory liver damage, Scd4 appears to play an important role in colitis and exerts protective effects in intestinal inflammation. The Scd4 deficiency leads to a higher permeability of the colon to *C. rhodentium* and a delayed cell migration. Further analysis are needed to explore the mechanisms of Sdc4-signalling in colitis.

A6.7 ALTERATIONS IN NAILFOLD VIDEOCAPILLAROSCOPY IN PATIENTS WITH GRANULOMATOSIS WITH POLYANGIITIS (WEGENER'S): AN OBSERVATIONAL STUDY

doi:10.1136/annrheumdis-2013-203220.7

Julia Uceda, Rosalía Martinez, María L Velloso, José L Marenco. *Rheumatology Department, Valme University Hospital, Sevilla, Spain*

Background Nailfold videocapillaroscopy (NFC), allows for the detection of changes in microcirculation. In the granulomatosis with polyangiitis (GPA) the existence of a defined pattern has not been found.

Objectives The main objective of our study was to detect the possible existence of a defined pattern in the microcirculation of the nailfold capillaries of patients with GPA. The second objective was to investigate the possible correlation between abnormalities found and systemic involvement.

Methods We identified 10 patients with a current mean age of 55.7 ± 16.5 years and predominantly female (60%). The mean age at diagnosis was 49.4 years. 70% had upper respiratory tract involvement, the same percentage had pulmonary involvement (cavitated nodules or alveolar haemorrhage), the cutaneous manifestations such as purpura or necrotic ulcers were present in 70%. About 40% had renal involvement (renal failure, proliferative glomerulonephritis), and 40% had peripheral neurological involvement. NFC was carried out by the same rheumatologist, on fingers 3 through to 5 of both hands using a ZUZI videocapillaroscopy, trinocular, dual illumination and zoom of 1 X 4 X.

Results Abnormalities of the microcirculation of nailfold capillaries were found in 8 of the 10 patients. Among the patients with this pathological microcirculation, 62.5% had structural alterations (tortuous capillaries), 50% presented with micro-haemorrhage (single or multiple), avascular areas were found in 37.5% and 75% showed lower capillary density. Neither capillary dilation nor the formation of new vessels were detected within the sample of patients.

Abstract A6.7 Table 1 Correlation between capilaroscopic finding with organ involvement

Organ involvement	Pathological capillaroscopy	Abnormal morphology	Bleeding	Avascular areas	Reduced capillary density	Expansion
Respiratory (7)	5	3	3	2	5	0
Renal (4)	3	3	1	1	3	0
Neurological (4)	3	1	1	0	3	0
Skin (7)	6	3	3	2	4	0

Conclusions We have observed, more frequent bleeding, avascular areas and reduced capillary density and these findings were not related with any specific organ involvement. There is one only study in GPA which communicates a high percentage of avascular areas. [1]

Reference

1. Anders HJ, Haedecke C, Sigl T, Krüger K. Avascular areas on nailfold capillary microscopy of Patients with Wegeners granulomatosis. *Clin Rheumatol*. 2000, 19(2):86–8.

A6.8 BIOLOGICAL THERAPIES IN JUVENILE IDIOPATHIC ARTHRITIS

doi:10.1136/annrheumdis-2013-203220.8

ML Velloso Feijoo, R Martinez Perez, J Uceda Montañes, JL Marenco de la Fuente. *Rheumatology Unit, Valme University Hospital, Seville, Spain*

Background Biological therapies have dramatically changed the prognosis for children with juvenile idiopathic arthritis (JIA). There are doubts about the possibility of discontinuing treatment once remission is achieved. We focus in this question in our series.

Objective To assess the efficacy and safety of these drugs in our series of patients with JIA.

Matherials and Methods We identified 9 children with JIA treated with biologic therapies, and we made a description of our experience.

Results The mean age was 14.55 ± 5.85, with a female predominance (66.7%). At diagnosis, mean age was 4.94 ± 2.9, and at the beginning of biological treatment of 8.77 ± 2.63. The median time