A5.18 IMPACT OF RITUXIMAB ON SYNOVIAL GENE EXPRESSION

doi:10.1136/annrheumdis-2013-203219.18

Yasser M El-Sherbiny, Sarah Churchman, Paul Emery, Edward M Vital, Frederique Ponchel. *Division of Rheumatic and Musculoskeletal Disease, Leeds Institute of Molecular Medicine, St James's University Hospital, Beckett Street, Leeds, UK*

Introduction Quality and duration of rituximab therapy response in rheumatoid arthritis have not been totally explained. In the synovium, three studies have indicated a relationship between higher plasma cell numbers at baseline and worse, or shorter lasting responses [1–3]. In this study we measured synovial expression of genes involved in B-cell biology, cell survival, cell trafficking and inflammation.

Methodology Synovial arthroscopic biopsies were collected and snap frozen from patients at baseline and 26 weeks after rituximab. Immunohistochemistry was performed for CD3, CD19, CD20cy, CD138 and CD68 and scored for positive cells/mm^2 using digital image analysis. The remainder of the tissue was used for RNA extraction. A 48 gene custom Taqman array was designed, including genes for cell lineage markers (CD19, CD20, CD3, CD138 & CD68), BAFF-APRIL system, immunoglobulins, cytokines, chemokines and adhesion molecules or cell trafficking. Informative data are available from 32 biopsies pre-Rituximab treatment and 23 post-treatment. Unsupervised clustering was performed to all gene expression to all patients pre and post rituximab.

Results Clustering of gene expressions revealed 3 main groups pre-rituxmab synovial biopsies, a definite cluster of B cell/survival factors signature associated low expression other genes synovial biopsies was associated with a higher disease activity (DAS28) at baseline and after treatment compared to the other two groups pooled together. This resulted in a lower rate of EULAR Good response.

Comparing EULAR responders and non-responders, there were trends to higher baseline CD20 gene expression in non-responders ($p = 0.079$) and greater reduction in CXCL13 ($p = 0.066$) and MMP ($p = 0.024$) in responders.

Lower expression of ICAM ($p = 0.021$), FGF ($p = 0.044$), CD20 ($p = 0.055$) and p53 ($p = 0.025$) and higher expression of APRIL ($p = 0.029$) at baseline was associated with normalisation of CRP after therapy. Furthermore, these patients also showed a significantly greater reduction in expression of CD4, CD55, CD68, CXCL12, EGF, FGF, ICAM, PECAM, STAT5, TGF-beta, APRIL and BAFF (all $p < 0.05$).

Conclusions Results point to key differences in synovial gene expression in patients with clinical response to rituximab, obviously in relation with genes involved in cell trafficking and survival.

References

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A5.19 IN AFRICAN PATIENTS WITH RHEUMATOID ARTHRITIS ACPA RECOGNISE CITRULLINATED FIBRINOGEN AND THE IMMUNODOMINANT EPITOPES BORNE BY THE FIBRIN PEPTIDES α36–50 AND β60–74, LIKE IN CAUCASIANS

doi:10.1136/annrheumdis-2013-203219.19

[1,2]L Nogueira, [1]M Cornillet, [3]M Singwe-Ngandeu, [4]S Viatte, [5,6]S Bas, [5,7]C Gabay, [1,2]G Serre. *[1]Laboratory of "Epidermis Differentiation and Rheumatoid Autoimmunity", UMR CNRS 5165, INSERM U 1056, Toulouse III University; [2]Laboratory of Cell Biology and Cytology, University Hospital of Toulouse; Toulouse, France; [3]Unit of Rheumatology, School of Medicine, University of Yaoundé, Cameroun; [4]Arthritis Research, UK Epidemiology Unit, University of Manchester; [5]Division of Rheumatology, University Hospitals of Geneva; [6]Department of Genetics and Laboratory Medicine; [7]Department of Pathology & Immunology; University of Geneva, School of Medicine, Genève, Suisse*

Background and Objectives Although most studies concerning the Rheumatoid Arthritis (RA)-specific antibodies to citrullinated proteins (ACPA) have been performed in populations of Caucasian ancestry, anti-CCP2 antibodies were demonstrated to be also good markers for RA in African patients. In one cohort from Cameroon, frequency of the HLA-DRB1 shared epitope (SE) alleles, although higher in RA patients than in controls, was lower than in Caucasian patients. Furthermore, known Caucasian non-HLA susceptibility genes did not confer the same risk in Africans.

The purposes of the present study were, first, to analyse in African patients with RA the autoantibodies to citrullinated human fibrinogen (AhFibA), then, to investigate to what extent the two citrullinated fibrin peptides α36–50 and β60–74, which bear the immunodominant epitopes, were recognised by the sera of these patients.

Methods AhFibA and autoantibodies to α36–50 and β60–74 were measured by ELISA in the serum of 56 consecutive RA patients with established disease from the Rheumatology unit of Yaoundé, Cameroon. 101 patients with other rheumatic diseases or healthy individuals were used as controls.

Results Using the 95%-specificity thresholds previously defined with a French cohort, 73% of the RA sera from African patients were positive for AhFibA *versus* 83% in French patients. The anti-β60–74 and anti-α36–50 autoantibodies were detected in 41/56 (73%) and 25/56 (45%) African RA sera *versus* 71% and 51% in French RA patients, respectively. Moreover, 38/41 (93%) of the AhFibA-positive sera recognised either α36–50 and/or β60–74, as previously observed in French patients. Finally, the mean AhFibA (OD = 1.28) and anti-β60–74 (OD = 1.32) titers were significantly higher in patients with at least one copy of the HLA-DRB1 SE (17/56, 30%) than in those without SE (OD = 0.64 and 0.60, respectively; $p < 0.005$ and $p < 0.02$), while the titer of anti-α36–50 autoantibodies did not differ.

Conclusions AhFibA are markers for RA also in African patients. In these patients, autoantibodies to α36–50 and β60–74 peptides are present in proportions similar to those found in Caucasians. Like in Caucasians, they account for almost all the reactivity to fibrinogen. HLA-DRB1 SE alleles, although less common among African RA patients, are associated with higher titres of AhFibA and of autoantibodies to β60–74, suggesting that SE alleles partly control the AhFibA production, besides still unknown factors. This study shows that even in different genetic backgrounds the ACPA response is a hallmark of RA, and strongly suggests that its fine specificity to citrullinated fibrin is identical in Caucasians and Africans.

A5.20 INTRAVENOUS IMMUNOGLOBULIN INDUCES FUNCTIONNAL SILENCING IN HUMAN B LYMPHOCYTES

doi:10.1136/annrheumdis-2013-203219.20

Sophie Hillion, Jean-François Seité, Carole Goutsmedt, Pierre Youinou, Jacques-Olivier Pers. *EA2216 Immunology & Pathology, Brest University and European University of Brittany, Brest, France*

Purpose/Objective Inappropriate B cell receptor (BCR) signalling contributes to the activation of auto-antibody-producing B cells in many autoimmune diseases. These diseases results from a break in B-cell tolerance which is normally maintained by the mechanisms of anergy, receptor editing and deletion. We previously showed that IVIg modulates many B cell responses through a sialic acid-CD22 lectin interaction (Séité, *Blood* 2010, Séité, *J Autoimmunity*, 2011). Here, we reasoned that IVIg could modulate B-cell fate and may render autoreactive B cells tolerant through induction of a functional unresponsiveness response to BCR stimulation.

Material and Methods Tonsilar B-cells were stimulated in the presence of different fractions of IVIg. We analysed subsequent responses to BCR stimulation by confocal microscopy, quantitative RT-PCR, FACS and Western-blot analysis.