1. Preclinical changes in immune-mediated inflammatory disease

A1.1 ANTI-CITRULLINATED PROTEIN ANTIBODY SPECIFIC Fc GLYCOSYLATION PATTERNS IN ARTHRALGIA PATIENTS

doi:10.1136/annrheumdis-2013-203214.1

¹AC Kempers, ¹HU Scherer, ¹Y Rombouts, ¹E Ewing, ²LA van de Stadt, ³MHJ Selman, ³AM Deelder, ¹TWJ Huizinga, ³M Wuhler, ²D van Schaardenburg, ¹REM Toes. ¹Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands; ²Department of Rheumatology, Jan van Breemen Research Institute/Reade, Amsterdam, The Netherlands; ³Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

Background/Objectives During rheumatoid arthritis, anti-citrullinated protein antibodies (ACPA) exhibit a specific, pro-inflammatory Fc glycosylation profile that is characterised by a low content of galactose and sialic acid residues. The absence of these sugars from the Fc-linked core glycan could influence the biological activity of ACPA during disease. As ACPA can be detected in sera several years before disease development, we hypothesised that a change in ACPA Fc-glycosylation might precede the onset of arthritis.

Methods Serum samples (n = 300) of patients with ACPA positive arthralgia (n = 184) were obtained at various time points. In this cohort, 96 patients developed arthritis after an average duration of 14.7 months of arthralgia. At the time of the onset of arthritis, patients were defined as having rheumatoid arthritis (RA, n = 51) based on the 1987 ACR criteria for RA, or undifferentiated arthritis (UA, n = 45). ACPA were isolated from serum samples by affinity purification, using cyclic citrullinated peptides as antigen. Purified ACPA-IgG and total serum IgG were digested with trypsin and resulting IgG1 Fc glycopeptides were analysed by mass spectrometry.

Results No significant change in Fc-glycosylation patterns was found between ACPA-specific and total serum IgG1 at the patients' first presentation with arthralgia (baseline). However, at diagnosis of arthritis, RA patients but not patients with UA exhibited increased hypogalactosylation of the ACPA and total IgG Fc fragments compared to healthy donors. The decrease of ACPA galactosylation levels at the Fc-tail occurred at 6 months before diagnosis and was significantly more pronounced at 3 months before diagnosis compared to total IgG.

Conclusions A decrease in Fc-galactosylation levels of ACPA occurred around 6 months prior to RA onset. Of interest, this ACPA hypogalactosylation was more pronounced than that of total IgG1, indicating that a more pro-inflammatory Fc-glycosylation pattern could be one mechanism driving inflammation in RA.

A1.2 FIBROBLASTS INFLUENCE LYMPHOCYTE RECRUITMENT AND MIGRATION DURING RESOLVING AND PERSISTENT ARTHRITIS

doi:10.1136/annrheumdis-2013-203214.2

^{1,3}Helen M McGettrick, ^{1,3}Andrew Filer, ^{1,3}Karim Raza, ²Gerard B Nash, ^{1,3}Christopher D Buckley. ¹Centre for Excellence in the Pathogenesis of Rheumatoid Arthritis; ²Centre for Cardiovascular Sciences, College of Medical and Dental Sciences; ³Systems Science for Health, University of Birmingham, Birmingham B15 2TT, UK

Background and Objectives Fibroblasts actively regulate the recruitment of leukocytes by endothelial cells (EC), acting in a pro- or anti-inflammatory manner depending on their site of origin. The phenotype of the fibroblast may be a critical determinant of whether leukocyte recruitment, and therefore inflammation, resolves or persists. Here we examined how synovial fibroblasts from different stages of arthritis influenced the recruitment of peripheral blood lymphocytes (PBL) and their onward migration.

Materials and Methods Fibroblasts were isolated from patients with resolving or persistent arthritis. Rheumatoid arthritis (RA) cohorts were categorised based on the stage of the disease at the time of sample collection: very early; newly presented but established or long established undergoing replacement surgery. Two forms of co-cultures were developed: (1) To assess effects on recruitment, EC and fibroblasts were cultured on opposite sides of porous philtres and incorporated into a novel flow chamber. PBL were perfused and observed as they bind to the EC surface. (2) To examine effects on migration, EC monolayers were formed on a philtre above a collagen gel in which fibroblasts were incorporated. PBL migration through the construct and their location within the gel were assessed. Conditioned media from co-cultures were collected and analysed by Luminex.

Results Fibroblasts from patients with RA increased the ability of EC to support PBL recruitment from flow in a disease duration-specific manner, with binding increasing from very early < established < replacement. However, levels of binding to very early RA co-cultures were similar to those observed when fibroblasts from non-inflamed or resolving tissue were incorporated. In the multi-cellular gel model, all fibroblasts, expected those from non-inflamed tissue, promoted PBL transendothelial migration but had no effect on entry into the gel construct. Interestingly, a greater proportion of PBL migrated into the lower half of the gel when fibroblasts from patients with very early and established RA were incorporated. Elevated levels of IL-6, IL-1 β , IL-8, Gro α and IP-10 were detected in the supernatants from RA co-cultures compared to resolving co-cultures. Resolving fibroblasts dramatically reduced the secretion of these soluble mediators by EC, suggesting they potentially have a suppressive effect.

Conclusions Collectively these initial data indicate that changes in the ability of fibroblasts to influence endothelial and lymphocyte behaviours may occur very early in the development of RA. Moreover, some of these changes are distinct from the phenotype exhibited by fibroblasts taken from non-inflamed tissue and acutely resolving arthritis.

A1.3 CITRULLINATION IN HEALTHY AND INFLAMED LUNG TISSUE AS A PRIMING SITE FOR AUTOIMMUNITY IN RA

doi:10.1136/annrheumdis-2013-203214.3

¹Elena B Lugli, ¹Anna Montgomery, ²Karin Lundberg, ³Ken Bracke, ³Guy Brusselle, ¹Patrick J Venables. ¹Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Muscular Diseases, University of Oxford, UK; ²Karolinska Institutet, Stockholm, Sweden; ³Ghent University, Gent, Belgium

Background Anti-citrullinated peptide/protein antibodies (ACPAs) are the key pathogenic autoantibodies in rheumatoid arthritis (RA). Because smoking is a risk factor for RA and ACPA often appear years before the onset of disease, it has been proposed that the lung may be a site for priming the ACPA response. Previous studies using immunohistochemistry suggested that smoking upregulates the expression of PAD2 and PAD4 with the resultant increased expression of citrullinated proteins. However these studies are limited by the availability of healthy lung tissue. In this study we used lung tissue taken at a distance from the primary tumour in lobectomy specimens and antibody reactivity to PAD2 and PAD4 and to two important precursor antigens in RA, alpha-enolase and fibrinogen, was defined by immunoblotting to ensure specificity.

Methods Lobectomy specimens from 40 subjects undergoing surgery for tumours or bronchiectasis (10 never smokers, 10 smokers without airflow limitation, 10 COPD ex-smokers and 10 COPD current smokers) were carefully dissected to remove a sample of uninvolved lung. The tissue samples were examined by immunoblotting with an anti-modified citrulline (AMC) antibody and scored for the