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CAPTURE OF IGA IMMUNE COMPLEXES AND ENRICHMENT IN IGA IG GENE EXPRESSION SUGGEST A ROLE FOR SYNOVIAL FCRL4+ B CELLS IN THE LINK BETWEEN MUCOSAL AND JOINT INFLAMMATION

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Introduction Increasing evidence points to the autoimmune process of rheumatoid arthritis (RA) originating at mucosal surfaces. Previous work from our group described a subset of B cells in the synovium and synovial fluid (SF) of RA patients distinguishable by their expression of Fc-like receptor 4 (FcRL4) and elevated expression of RANKL, indicating a unique pathogenic function.^{1,2} B cells expressing FcRL4 were originally described as a distinct memory B cell subset in human tonsils where they are localised in the epithelium.³ We have recently shown in RA that they are enriched in clones recognising citrullinated autoantigens.³ Recent *in vitro* work suggested that FcRL4 is a low affinity receptor for aggregated IgA.⁴

Objectives

- Investigate the interactions between IgA and FcRL4 and FcRL4+ B cells using tonsil and SF B cells, and transfected cell lines.
- Examine the distribution of Ig classes by flow cytometry and PCR.

Methods Mononuclear cells were isolated from SF (n=10) and tonsil, labelled for CD19, FcRL4, IgA, and IgG and analysed by flow cytometry. In experiments identifying BCRs and relative loads of surface bound IgA; SFMCs were washed in an acidic buffer to remove receptor-bound proteins before staining. Heat-aggregated purified human IgA was added to assess IgA binding to FcRL4 +B cells. Single B cells were sorted from SF of RA patients and their constant region genes probed for identification of their Ig subclass by PCR.

Results We show that *ex vivo* SF FcRL4⁺ B cells have a significantly higher load of IgA bound to their surface (p=0.0001) than FcRL4⁻ B cells. Following *in vitro* removal of surface bound IgA, FcRL4 +B cells bind heat-aggregated IgA (p=0.03 vs control). We also demonstrate that a significantly higher proportion of FcRL4⁺ B cells use IgA BCRs (p=0.0061) by flow cytometry, and by probing constant region genes by PCR an enrichment for Ig genes for coding the IgA1 isotype (p=0.009) was found. Furthermore, three out of eight of the antibodies recognising citrullinated peptides had been cloned from FcRL4⁺ IgA⁺ B cells.

Conclusions In conclusion, their specificity for citrullinated antigens, ability to capture IgA immune complexes through via FcRL4 and enrichment in IgA1 subclass expression, suggest a role for FcRL4 +B cells in the mucosal origin of joint inflammation.

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ABSTRACT WITHDRAWN

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CELL SURFACE SIALYLATION OF B-CELL SUBSETS FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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Introduction B-cells have a well-established role in the pathogenesis of rheumatoid arthritis (RA), illustrated by the successful treatment of RA with B-cell depletion therapy.¹ Sialic acid (SA) is a 9-carbon backbone sugar involved in many immune system functions including cell migration, adhesion and activation.² In RA, sialylation of the B-cell surface, including membrane bound IgM, has been found to differ from healthy control B-cells. These changes have been attributed to alterations in antibody glycosylation which lead to the highly inflammatory phenotype of disease-specific autoantibodies.³ Recently it has been shown that surface glycosylation of regulatory T-cells is altered by activating stimuli – suggesting functional consequences for cell surface glycans.⁴ Functional effects of surface glycans in B-cells in RA have not been well established.

Objectives The aim of this study is to investigate surface sialylation of B-cells from patients with RA at different stages of disease, and to examine functional consequences of B-cell surface sialylation.

Methods Sialylation of peripheral blood mononuclear cells isolated from 7 patients with either ‘pre-RA’ or RA (3 patients with ACPA+ ‘pre-RA’, 1 patient with a diagnosis of new onset, ≤12 months symptom duration, and 3 patients with established disease) was analysed by flow cytometry using biotinylated Sambucus Nigra (SNA) Lectin, to bind to α2,6 SA, with PE-conjugated streptavidin. MESF beads were used to standardise the measurement of fluorescence intensity. B-cells were activated in culture with anti-CD40 and anti-IgM.

Results Initial data suggests that B-cell surface sialylation may be higher in patients with pre-RA (MESF: 13765±170) or new onset RA (13094) than in patients with established RA (12431.3±339). Sialylation also tended to be higher on plasmablasts (1396±991) than on memory (13067±496) or naïve B-cells (13127±710). Upon *in vitro* activation with anti-CD40 and anti-IgM, sialylation tended to decrease in plasmablasts (20042±679 vs 16200.5±1261).

Conclusions Preliminary results suggest that B-cell surface sialylation may vary according to stage of disease and B-cell subset. Sialylation may also be related to B-cell activation. Further investigations will be carried out to better understand variations in surface sialic acid and examine the relationship between B-cell function and surface sialylation.

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