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LYMPHOID TISSUE MACROPHAGES EXPRESS PEPTIDYLARGININE DEIMINASE 2 AND 4: NEW IMPLICATIONS IN CITRULLINATION AND ANTI-CITRULLINE ANTIBODIES PRODUCTION IN AUTOIMMUNE ARTHRITIS

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Introduction The role of citrullination in breach of tolerance in rheumatoid arthritis (RA) is well established, but the mechanisms linking protein citrullination and the generation of anti-citrullinated protein/p peptide antibodies (ACPA) are poorly understood. We have recently reported a critical role of peptidylarginine deiminase (PAD) 4 expression and extracellular trap formation by neutrophils in ACPA production in RA.¹ In lymphoid tissues, neutrophils are scarce, whereas macrophages are abundant antigen presenting cells where they regulate T and B cell responses and play fundamental roles in the chronicity of RA.

Objectives To test our hypothesis that antigen presenting cells (APCs) in lymphoid tissues express PADs and contribute to protein citrullination and ACPA production.

Methods We tested our hypothesis in collagen induced arthritis (CIA) mice and synovial biopsies from RA patients. PAD4 and 2 expression was assessed in the spleens and lymph nodes of CIA mice and their distribution was revealed by immunohistochemistry (IHC). The impact of macrophage depletion on lymphoid tissues' PAD activity, citrulline content, and serum ACPA levels was analysed; and the effect of macrophage stimulation on extracellular trap formation, PADs expression and release was investigated. Additionally, PAD4 expression in RA synovia was verified by IHC; and the correlation of PAD4 with macrophage-related genes was studied by RNA-Seq analysis.

Results Our results indicated that serum ACPA were detectable in CIA sera before clinical arthritis; and PAD4 and 2 were expressed in the draining lymph nodes and spleens as shown by qPCR and Western blotting. In addition, PAD4 and 2 labelling specifically co-localised with macrophages but not other APCs. Moreover, PAD4 and -2-expressing tingible body macrophages (TBM) were associated with citrulline-retaining follicular dendritic cells (FDCs) in the germinal centres (GC). *In vitro* TLR activation of macrophages enhanced PAD4 and 2 expression; and stimulation of TBMs with TNF- α induced Macrophage Extracellular Trap formation (METosis), release of apoptotic bodies, and DNA-associated citrullinated proteins. Whereas retention of citrullinated proteins on FDCs induced ACPA production in *in vitro* GC reactions, macrophage depletion in CIA mice significantly reduced citrullinated proteins in lymphoid tissues and inhibited serum ACPA levels. In RA synovia, PAD4 colocalized with CD68 macrophages and was expressed in TBMs associated with citrulline-retaining FDCs in ectopic lymphoid structures. RNA-Seq analysis of PAD4 expression in RA synovial biopsies identified significant positive correlation with 279 genes associated with macrophage activation and function.

Conclusions In conclusion, this work reports new mechanisms by which macrophages contribute to citrulline-induced autoimmunity. Therapeutic targeting of these mechanisms would inhibit citrullination, ACPA production and progression of autoimmune diseases.

REFERENCE

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Disclosure of interest None declared

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TARGETING IL-10 PRODUCING B CELLS IN RHEUMATOID ARTHRITIS AND PRIMARY SJÖGREN SYNDROME IS PROMISING TO INCREASE REGULATORY T CELLS BUT NOT TO DECREASE PRO-INFLAMMATORY T CELLS

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Introduction The role of regulatory B cells in tolerance has been established in both preclinical and clinical studies. There is no specific marker for human regulatory B cells. They have been mainly ascribed to CD24^{hi}CD38^{hi} and C24^{hi}CD27⁺ B cells which can increase regulatory T cells and decrease pro-inflammatory Th1 and TNF α -producing T cells. However, in patients with auto-immune diseases, such as Rheumatoid Arthritis (RA) or Sjögren Syndrome (pSS), CD24^{hi}CD38^{hi} and C24^{hi}CD27⁺ B cells lose their regulatory functions. It is thus needed to find a better marker of Breg cells that correlate with regulatory functions in both healthy controls and patients. As Breg cell functions are mainly mediated by IL-10, some authors use IL-10 production to define Breg cells, which are then called B10⁺ cells. We previously showed that B10⁺ cells are decreased in RA and negatively correlated with the disease activity, suggesting a key role of regulatory B10⁺ cells in autoimmune diseases. Before using B10⁺ cells as a therapeutic strategy it is necessary to precisely determine their functions. However, it remains unknown if any B cell secreting IL-10 has regulatory functions and which ones.

Objectives To assess the functions of B10⁺ cells, compared to non-IL-10 producing B cells (B10^{neg} cells) on T cell differentiation in healthy controls (HC) and in patients with autoimmune diseases, namely RA and pSS.

Methods B10⁺ cells were induced by a 24 hour stimulation with CpG and sorted using isolation kits and flow cytometry. We evaluated their effect on naïve T cell differentiation into Th1, TNF α -producing T cells, Treg cells and IL-10 producing T cells (Tr1). We characterised the interaction between B10⁺ cells and T cells, using blocking antibodies against IL-10, CD80, CD86, PD1 and transwell experiments.

Results B10⁺ cells of HC converted more naïve T cells into Treg cells and Tr1 than B10^{neg} cells (p=0.03 and p=0.02). Blocking either IL-10 secretion or cellular contacts between B10⁺ cells and T cells abolished the induction of Treg cells. Surprisingly, conversely to CD24^{hi}CD27⁺ B cells, B10⁺ cells failed to decrease Th1 and TNF α -producing T cell conversion, compared to B10^{neg} cells. This might be due to a different expression of PDL1 and PDL2 between B10⁺ cells and CD24^{hi}CD27⁺ B cells. Similarly to HC, B10⁺ cells from RA and pSS patients induced more Treg cells compared to B10^{neg} cells (p=0.02 in RA; p=0.03 in pSS). However, conversely to HC and pSS-B10⁺ cells, RA-B10⁺ increased the conversion of naïve T cells into Th1, compared to B10^{neg} cells (p=0.033).