SATURDAY, 16 JUNE 2018

Innate immunity in rheumatic diseases

SAT0001

MECHANISM AND SIGNIFICANCE OF COMPLEMENT C3 RECEPTOR IN COLLAGEN-INDUCED RHEUMATOID ARTHRITIS MICE MODEL

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Background: Rheumatoid arthritis (RA) is a kind of chronic autoimmune disease, mainly manifested as small joint synovitis. Disease progression appears joint swelling, bone and cartilage damage, deformity and activity limited. The etiology of RA is still unclear and is generally considered to be an immune-mediated inflammatory disease. The activation pathway and regulation function of the complement system have become the hotspot of the research of RA pathogenesis. Previous studies have found that C3-/- mice had lower levels of antibodies to collagen in the Type II collagen-induced rheumatoid arthritis (CIA model), and the molecular mechanism is unclear. The small fragment C3a and large segment iC3b produced by C3 activation are the main effect products, and their biological effects are performed by combining with the specific receptor, C3aR and CD11b respectively.

Objectives: To investigate the mechanism of C3a and C3b, the cleavage products of complement C3 in rheumatoid arthritis, binding to their corresponding receptors, the signalling pathway of complement activation and the effect on arthritic conditions.

Methods: This study was intended to establish a CIA model on C3aR knockout and CR3 knockout mice (C3aR-/- or CD11b-/-) to investigate the effect of complement C3a-C3aR signalling and iC3b-CR3 signalling on rheumatoid arthritis. Methods using C57BL/6 background transgenic mice (Gifted by King's College London). Mice were divided into 3 groups according to the experimental mouse strains: C3aR-/- group, CD11b-/- group and WT control group. The clinical score of the joints in each group was measured after the establishment of the CIA model through collagen induction. Moreover, joint specimens were collected for pathologic grading. Besides, the level of CD4 +T cell, CD8 +T cell, Th17/Treg ratio and the level of IFN-gamma of NK cell in mouse spleen were detected by flow cytometry.

Results: 1. The clinical score of C3aR-/- group was slightly lower than that of WT group, and the clinical score of CD11b-/- group was significantly higher than that of WT group; 2. Pathological score (12 points): The CIA scores of CD11b-/- group, C3aR-/- group and WT group were 9.35±0.75, 4.81±0.63 and 5.85±0.55 respectively. The CIA scores of CD11b-/- group was significantly higher than that of WT group, which were consistent with clinical score; 3. Through the flow cytometry detection, compared with the WT group, CD4 +T cell, CD8 +T cell and Th17 percentage increased significantly, and Treg cell decreased. In addition, the secretion of IFN-gamma of Splenic NK cell was significantly reduced.

Conclusions: iC3b as well as C3a could bind to their respective complement receptor, and express different influence in the immune mechanism of RA. The iC3b-CD11b signalling has a protective effect in RA, while the C3a-C3aR signalling has an inflammatory aggravation effect. Through this study, it helps us to invent the drug related to complement components and their receptors, and further be used in the clinical treatment of RA.

Disclosure of Interest: None declared

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SAT0002

THE INTRACELLULAR ITAM TYROSINES OF FC RECEPTOR GAMMA-CHAIN ARE CRITICAL FOR EXPERIMENTAL AUTOIMMUNE ARTHRITIS

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Background: Activating Fcγ-receptors on neutrophils associate with the Fc-receptor γ-chain (FcRγ), an immunoreceptor tyrosine-based activation motif (ITAM) containing transmembrane adapter molecule. Previously, we carried out *in vitro* experiments that showed not only a chaperon-like function of FcRγ, but also a signalling role through its intracellular ITAM-tyrosines.

Objectives: Here, we investigated the participation of these tyrosines in an auto-antibody-induced arthritis model (in the KBxN serum transfer arthritis, for the

development of which neutrophils and Fc-receptors are essential) using wild type and ITAM tyrosine mutant (Y65F/Y76F) transgenic mice.

Methods: The experimental animals expressed wild type or ITAM tyrosine mutant (Y65F/Y76F) Fc-receptor γ-chain on the FcRγ^{-/-} genetic background. The arthritis was initiated by a single intraperitoneal injection of control or arthritic serum. The severity of joint inflammation was followed by clinical scoring, measuring ankle thickness changes and detecting joint dysfunction. Homozygous transgenic mice were identified by quantitative PCR.

Results: Compared to wild type mice, FcRγ knockout animals failed to exhibit a measurable joint inflammation. Surprisingly, the arthritis could not develop in wild type FcRγ transgene heterozygous mice. To enhance the expression level of the wild type transgene (which was approximately one third of the expression of the mutant transgene), we crossed our wild type transgene heterozygous mice with each other. As a consequence, wild type FcRγ transgene homozygous mice on the γ-chain knockout background were able to undergo arthritic development. In contrast, FcRγ knockout mice carrying the ITAM-mutant FcRγ transgene (both at heterozygous and homozygous forms) were fully protected from the development of arthritis despite of comparable neutrophil cell surface Fcγ receptor expressions.

Conclusions: Our *in vivo* experiments show that the intracellular Fc receptor γ-chain ITAM tyrosines play a critical role in the initiation and progression of an auto-antibody-induced experimental arthritis model, confirming a signalling, rather than just a chaperon-like function of the molecule.

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SAT0003

SYNOVIAL TISSUE CD1C+ DENDRITIC CELLS IN RHEUMATOID ARTHRITIS EXPRESS HIGH LEVELS OF THE EPIGENETIC REGULATOR OF INFLAMMATION, MICRORNA-155 AND INFLAMMATORY CYTOKINES

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Background: Dendritic cells (DCs) direct the immune response against pathogens while maintaining self-tolerance by instructing T/B cells in lymphoid organs and peripheral tissues. However, their aberrant activation can lead to chronic inflammation and autoimmunity. Based on the distinct transcriptomics, function and distribution, DCs can be broadly categorised as plasmacytoid and myeloid (conventional) DCs. Based on recent single cells sequencing and secretome data, can be divided into CD141⁺ DCs (DC1), DC2_A (DC2) defined as "CD1c^{high}CD32B^{high}CD36^{neg}CD163^{neg}" and DC2_B (DC3) defined as "CD1c^{low}CD32B^{neg}CD163^{high}CD36^{high}". In addition, populations of CD1c⁺CD141⁺CD16⁺ DC (named DC4), that shares some gene expression with CD16⁺ monocyte and inflammatory DC (infDC). Most to date studies on Rheumatoid Arthritis patients investigated DCs in circulation or in synovial fluid (SF). This provided important insight into epigenetic changes in DC-precursors before they enter synovial tissue, e.g. RA blood CD1c⁺ have deregulated microRNA-34a driven epigenetic control of anti-inflammatory Axl pathway² or into the influence of inflammatory milieu on DCs, respectively. However, neither (peripheral blood) PB or SF are major sites for DC regulated T/B cell activation; instead, DCs control immune responses in the appropriate structures of lymph organs and tissues.

Objectives: In this study, we sought to investigate myeloid DCs in synovial tissue with the prospect of better understanding their role in driving autoimmunity in RA.

Methods: We developed a flow cytometry sorting strategy to characterise the phenotype of distinct myeloid DC subsets in multiple biological compartments (PB, SF and synovial tissue). Synovial tissue (ST) biopsies (RA n=9; Psoriatic arthritis n=3) were digested with liberase prior the analysis. Peripheral blood DCs (RA n=19, Psoriatic arthritis n=16, healthy donors n=12), and SF DC (n=3) were used as comparators. Synovial tissue, SF and PB DCs were sorted and micro-RNAs, pro-inflammatory and regulatory cytokine expression analysed by amplified qPCR. In addition, DCs were mapped in synovial tissue in RA (n=8), PsA (n=7) and control non-inflammatory OA (n=5) by immunohistochemistry.