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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 autoantibody-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 autoantibody-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 pathway[2] 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 microRNAs, 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.

Results: Myeloid DCs are scarce in control non-inflammatory OA synovial tissues and their number increased substantially in PsA and RA tissues. Phenotyping data revealed that all myeloid DC subsets can be present in inflamed RA and PsA synovium. However, CD1c$^+$ DC populations (DC2/DC3) were the most abundant in RA synovial tissues and the gene expression analysis of CD1c$^+$ sorted from RA synovial biopsies showed an increase in the expression of epigenetic regulator of inflammatory response miR-155 and IL-6, TNF and IL-23 as compared to circulating cells.
Conclusions: CD1c$^+$DCs from RA synovial tissues had epigenetically regulated activated phenotype (miR-155 and miR-34a^2) that through the production of cytokines could maintain tissue activation of autoreactive Th1 and Th17 cells and contribute to inflammation.

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SAT0004 INTRA-ARTICULAR INJECTION OF ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS REDUCE EXPERIMENTAL OA PATHOLOGY VIA IL-1B-MEDIATED REALLOCATION AND ENHANCED PHAGOCYTOSIS OF POLYMORPHONUCLEAR LEUCOCYTES

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Background: Injection of adipose-derived mesenchymal stromal cells (ASCs) into knee joints after induction of experimental inflammatory osteoarthritis (CiOA) reduces development of joint pathology.[1] This protection is only achieved when ASCs are applied in early CiOA, which is characterised by synovitis and high levels of S100A8/A9 and IL-1β, suggesting that inflammation boosts the protective effect of ASCs.[2]
Objectives: To examine the role of synovitis in ASC-mediated amelioration of CiOA pathology.
Methods: CiOA was induced by intra-articular collagenase injection. Knee joint sections were stained with haematoxylin/eosin and immunolocalization of polymorphonuclear leucocytes (PMNs) and ASCs was performed using antibodies for NIMP-R14 and CD271, respectively. Chemokine expression induced by IL-1β or S100A8/A9 was assessed with qPCR and Luminex. Migration of PMNs through transwell membranes towards ASC-conditioned medium (CM) was examined using flow cytometry. ASC-PMN co-cultures were analysed microscopically and with Luminex. Phagocytic capacity of PMNs was measured with labelled zymosan particles.
Results: Intra-articular injection of saline in knee joints of day 7 CiOA induced a flare already after 6 hours, characterised by particularly PMNs scattered throughout the synovium. Although ASC injection resulted in comparable numbers of PMNs, these cells however, were clustered around ASCs. IL-1β-stimulation of ASCs *in vitro* strongly increased expression of PMN-attracting chemokines KC, CXCL5, and CXCL7, whereas S100A8/A9 did not. Migration of PMNs towards CM of IL-1β-stimulated ASCs (IL-1β-CM) was significantly enhanced (2.9-fold increase) when compared to CM of non-stimulated ASCs (NS-CM). After 6 hours co-culturing PMNs with IL-1β-stimulated ASCs, the number of clustered PMNs per ASC was significantly increased. Interestingly, association of PMNs with ASCs significantly diminished the release of KC protein by ASCs (69% lower after 24 hour), and also strongly reduced the release of S100A8/A9 protein by the PMNs. Moreover, phagocytic capacity of PMNs was strongly enhanced after priming with CM of IL-1β-stimulated ASCs.
Conclusions: Local application of ASCs in inflamed CiOA knee joints results in attraction and clustering of PMNs with ASCs in the synovium, which is likely mediated by IL-1β-induced up-regulation of chemokine release by ASCs. This results in lowered S100A8/A9 levels and enhanced phagocytic capacity of PMNs, enabling the clearance of debris to attenuate synovitis and promote tissue repair.

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SAT0005 INTERLEUKIN-33 AMELIORATES MURINE LUPUS VIA INDUCTION OF REGULATORY T CELLS AND M2 MACROPHAGE POLARISATION

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Background: The levels of IL-33, a Th2 promoting cytokine, and the soluble form of its receptor ST2 were reported to be elevated in serum of patients with active systemic lupus erythematosus (SLE), suggesting a role of the IL-33/ST2 axis in the pathogenesis of SLE.
Objectives: This study aims to examine the effect of IL-33 in disease severity of murine lupus.
Methods: IL-33 was injected intraperitoneally 3 times per week to pre-diseased MRL/lpr mice aged 12 weeks for 6 weeks. Control group was given 1% BSA injection. Urine protein was monitored weekly by albustix and protein assay. Immunophenotyping of splenocytes was examined by flow cytometry. Splenic CD11b +monocytic cells were isolated by microbeads for mRNA examination.
Results: IL-33-treated mice (n=9) developed significantly less proteinuria compared to BSA-treated group (n=9). Kidney histology of the IL-33-treated group showed remarkably less mesangial deposit, diffuse proliferative glomerular changes and crescents, and had significantly lower renal composite score compared to controls (median 2.0 vs 9.9, p<0.001). Kidneys of these mice expressed lower mRNA levels of TNF-α (32.1±14.7 vs 77.0±27.8, p<0.001), IL-6 (median 0.6 vs 4.7, p=0.003), IL-1β (31.1±10.1 vs 77.8±24.6, p<0.001) and iNOS (p=0.006). Immunophenotyping of splenocytes showed significantly increased CD4 +CD25+regulatory T (Treg) cells (4.0%±1.2% vs 2.2±0.2%, p<0.001) that expressed remarkably higher Foxp3 (76.0%±5.0% vs 59.3±12.6%, p=0.002). Splenic extracts showed predominant Gata3 (0.37±0.20 vs 0.12±0.09, p=0.01) and Foxp3 (0.42±0.16 vs 0.17±0.11, p=0.002) mRNA in IL-33-treated mice. These Treg cells expressed high cell surface ST2 (8.9%±2.7% vs 4.5±2.0%, p=0.008). There was significant expansion of splenic CD11b+population in IL-33-treated mice (17.8±10.5 vs 8.8±3.0, p=0.01) that expressed significantly higher CD206 (5.2%±0.9% vs 2.9±0.9%, p=0.002). Isolated splenic CD11b+cells expressed significantly higher mRNA of Arg1, FIZZI and Ym-1 and IL-10 (all p=0.01) with reduced expression of iNOS (p=0.02). Kidney extracts of IL-33 treated mice also had elevated mRNA levels of M2 markers including Arg1 (median 199.8 vs 36.1, p=0.004) and FIZZI (median 25.0 vs 2.7, p<0.001) and reduced MCP-1 (12.7±6.5 vs 35.1±12.0, p<0.001). There was also significantly higher levels of mRNA of Foxp3 (median 43.0 vs 20.8, p=0.006) and Gata 3 (1.7 ±0.5 vs 0.9±0.5, p=0.008) but lower Rorc (2.6±1.0 vs 3.8±0.8, p=0.008) and Tbx21 (12.6±6.0 vs 29.6±13.7, p=0.003) in the kidneys.
Conclusions: Exogenous IL-33 led to significantly less proteinuria and renal inflammation. These mice had significantly higher splenic Treg cells with prominent Foxp3 expression. Isolated CD11b+cells from spleen and kidney extracts demonstrated mRNA levels of M2 macrophage polarisation.
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SAT0006 P2X7 RECEPTOR IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE). EXPLORING A NOVEL PATHOGENETIC PATHWAY IN LUPUS RELATED SEROSITIS

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Background: Recent studies have focused attention on the involvement of innate immunity and in particular on the activation of NLRP3 inflammasome by purinergic signalling mediated by P2 × 7 receptor (P2 × 7R), in SLE pathogenesis.[1] Serositis are typical SLE manifestations often associated with increased inflammatory indices and promptly responding to colchicine whose action could be mediated by its effect on microtubules during P2 × 7R assembly.
Objectives: To explore the role of innate immune system in SLE evaluating expression and activity of P2 × 7R and NLRP3, comparing patients with positive and negative history of serositis with healthy control subjects (HC).