OP59. THE VALUE OF INTERLEUKIN-17 SERUM LEVEL IN RHEUMATOID ARTHRITIS IMMUNOPATHOGENESIS

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Background: Interleukin (IL)-17 is the main Th1 cytokine, produced by activated T-lymphocytes. The potential IL-17 value in rheumatoid arthritis (RA) pathogenesis consists of its independent inflammatory response induction and mediated stimulation of proinflammatory factors synthesis resulting in joint destruction. The aim of study was to determine the role of IL-17 in immuno-inflammatory/autoimmune reactions development and to reveal IL-17 serum level associations with clinical and immunological characteristics of RA.

Methods: 50 patients with early RA (disease duration < 12 month) and 15 healthy individuals were examined. All patients underwent complex clinical and laboratory examination. The immunological investigation included lymphocyte count and its subpopulation composition determination using monoclonal antibodies; detection of circulative immune complex level was done by Digeon method, IgG, IgM, IgA - by Manchini method. The serum concentrations of CRP(DAI, USA), sCD40L, matrix metalloproteinases (MMP)-3 (Bender MedSystems, Austria), IL-17 (Biosource, Belgium), rheumatoid factor (RF) (vKекторbest, Russia), anti-CCP antibodies (Aaxies-Shield Diagnostic, UK) were revealed using ELISA immunoassay.

Results: On the base of IL-17 serum level patients were divided in two groups: group1 (n = 22) were patients with normal IL-17 serum level and group2 (n = 28) were those with high IL-17 serum level. In the group2, the rate of patients’ pain assessment by visual analogue scale (67.3 ± 7.2 vs 32.8 ± 4.6; P = 0.001), tender (16.7 ± 2.0 vs 8.4 ± 1.1; P < 0.01) and swollen (12.3 ± 2.3 vs 3.9 ± 0.8; P = 0.01) joint count, DAS28 (5.0 ± 0.4 vs 2.8 ± 0.2 P < 0.01) were significantly higher compare to group1.

It was found that in group2 the higher T-lymphocyte amount (CD3+) was due to CD4 higher quantity, at the same time CD8 amount was significantly lower in the group2 (3.4 ± 0.3 U/ml vs 8.4 ± 0.8 U/ml, P < 0.05). The percentage of CD8+ cells (68 ± 46) compared with OA patients (55.4 ± 15%, n = 17, P = 0.05), HC (45.3 ± 8%, n = 15, P = 0.04) and RATNF patients (47.4 ± 15%, n = 21, P = 0.04). In addition, the expression of CD28+ (3%, RAMTX vs 7.2 ± 1.1% HC; P = 0.05) and IgM RF (r = 0.41; P < 0.05) were significantly lower in the group2.

Conclusion: This study indicates that although the percentage of CD3+ cells in RA patients is significantly higher compared to healthy individuals, the percentage of CD8+ cells is significantly lower. The CD8+ cells number in RA patients is significantly lower compared to OA and HC patients. Further investigation of CD8+ cells number in RA patients may be the potential target of RA patients’ management.

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expression and CSR. In addition, we dissected the molecular basis of stromal cell/B cell interactions with particular emphasis on the role of toll-like receptors (TLRs) signaling and B cell survival/proliferation factors.

Methods: mRNA and protein expression of B cell survival factors BAFF and APRIL in RA SF and OASF stimulated with TLR2, TLR3 and TLR4 ligands was assessed by Taqman PCR (QT-PCR) and ELISA, respectively. Un-switched IgD+ B cells were isolated from human tonsils using magnetic cell sorting. Isolated B cells were co-cultured via transwell or cell-cell contact with RA SF/OASF for 24 h and 72 h in the presence or absence of TLR ligands and with or without BCMA-Ig as a selective binder of soluble BAFF/APRIL. AID mRNA expression and IgM/A/G production were measured to assess functional activation of B cells. In addition, IγCγ1 and IγCγ2 circular transcripts (CT, molecular by-products of ongoing CSR from IgM to IgG and IgM to IgA, respectively) were assessed by rt-PCR.

Results: In vitro stimulation of TLR3 and to a significantly lesser extent TLR4, but not TLR2 on RA SF led to strong induction of BAFF (~1,000-fold increase with TLR3) and APRIL mRNA expression. In response to TLR3, BAFF was time-dependently released in the supernatant of RA SF (~300 pg/ml) and, to a lesser extent, OASF. TLR3 stimulation of RA SF in co-culture with B cells strongly enhanced AID expression, ongoing CSR to IgG, but not IgA, as shown by detection of IγCγ1 CT and release of IgG. By contrast, TLR4 stimulation alone had no direct effect on B cells. Conversely, blockade of soluble BAFF/APRIL by BCMA-Ig inhibited TLR3-induced AID-dependent production of AID mRNA, IγCγ1 CT as well as the secretion of IgG.

Conclusions: We demonstrated that RASF are able to release high levels of B cell survival factors upon TLR3 stimulation at both mRNA and protein level. The level of these factors was functional, as demonstrated by the capacity of RA SF to directly modulate AID expression, CSR and production of class-switched antibodies in co-cultured un-switched B cells. This effect was abrogated by blockade of soluble BAFF and APRIL. Overall, these data strongly suggest a dual role for TLR3-1 and TLR4 in cell-cell communication and CSR by RA SF, as measured by APRIL in sustaining functional B cell activation and antibody production.

Disclosure statement: All authors have declared no conflicts of interest.

OP62. QUANTIFYING IN VIVO FLUORESCENCE IMAGING IN MURINE ARTHRITIS BY TARGETING E-SELECTIN

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Background: In vivo molecular optical imaging can delineate the molecular and cellular biologic processes that are occurring at the tissue and molecular level. E-Selectin, a leucocyte adhesion molecule expressed on activated endothelium, is upregulated by TNFα and increased in RA. Furthermore, radiolabelled Fab(ab)’2 fragment of anti-E-selectin monoclonal antibody demonstrates increased specificity compared with conventional technetium-oxidonitrile scanning in patients with RA. Collagen-induced arthritis (CIA), an animal model of RA, has been widely used to study the pathogenesis of arthritis and to identify new therapies for RA, including anti-TNFα. Pro-inflammatory cytokines, such as TNFα and IL-1, are expressed in the arthritic joints of both murine CIA and RA. This study aimed to demonstrate and quantify E-Selectin targeted fluorescent imaging in vivo in a model of paw inflammation following local injection of murine TNFα and subsequently in CIA as a model of RA.

Methods: Anti-murine E-selectin and isotype control antibodies were cultured from hybridoma cell lines and labelled with Dylight 750 nm and 787 nm. PBMCs and SF macrophages were cultured in the presence of patients with inflammatory and non-inflammatory joint disease and synovial tissue samples were derived from biopsy and arthroplasty specimens. PBMCs and SF macrophages were cultured in the presence of inflammation mediators (TNFα, IL-1, IL-6 and IL-17) ± TNFα and ± OPG. Osteoclast formation was assessed by TRAP staining and osteoclast activity by lacunar resorption. TSG-6 expression in synovial tissue was assessed immunohistochemically.

Results: Our data show that:
1) TSG-6 is expressed by osteoclasts and in the synovium of RA patients; 2) TSG-6 and OPG have synergistic effects on the inhibition of RANKL-mediated bone resorption; 3) the inflammatory cytokines TNFα, IL-1 and IL-6 but not IL-17 induce TSG-6 production by osteoclast precursors (PMBCs) and 4) induction of TSG-6 expression in PMBCs correlates with an inhibition of osteoclast-mediated lacunar resorption, suggesting an autocrine control of osteoclast activity by TSG-6.

Conclusions: In the presence of inflammatory cytokines, osteoclast precursors produce TSG-6 at concentrations that are sufficient to inhibit osteoclast activity and reduce lacunar resorption. This may represent an autocrine mechanism to limit the degree of bone resorption during joint inflammation. Although in the complex milieu of inflammatory synovial fluid, this mechanism is insufficient to prevent local erosion, the chondroprotective, anti-inflammatory and anti-resorptive effects of TSG-6 make it a potential therapeutic option.

Disclosure statement: All authors have declared no conflicts of interest.

OP63. TSG-6: AN AUTOCRINE REGULATOR OF INFLAMMATORY JOINT DISEASE?

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Background: TSG-6, the secreted product of TNF-stimulated gene 6, is present in the cartilage, synovium and synovial fluids of RA patients. It is constitutively expressed by RA synovioctyes, where its production may be co-regulated by TNFα, IL-1 and IL-17. Animal models indicate a protective role for TSG-6 in inflammatory joint disease, with evidence of both anti-inflammatory and chondroprotective effects in mice with collagen- and antigen-induced arthritis. Recent in vitro studies have indicated an additional anti-resorptive effect of TSG-6, possibly via an interaction with RANKL, the major regulator of osteoclastogenesis. TSG-6 has a similar potency to OPG (the soluble decoy receptor for RANKL) but, unlike OPG, inhibits only osteoclast activation and not formation. The fully-humanized monoclonal antibody against RANKL, denosumab, has been shown to reduce erosion scores in clinical trials of RA, but its utility may be diminished by its lack of anti-inflammatory properties. This study sought to determine whether (i) TSG-6 acts synergistically with OPG in inhibiting osteoclastic bone resorption, (ii) TSG-6 is produced during cytokine-mediated osteoclastogenesis and (iii) TSG-6 can regulate the process of osteoclastogenesis in patients with inflammatory joint disease.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from synovial fluid (SF) macrophages derived from patients with inflammatory and non-inflammatory joint disease and synovial tissue samples were derived from biopsy and arthroplasty specimens. PBMCs and SF macrophages were cultured in the presence of inflammation mediators (TNFα, IL-1, IL-6 and IL-17) ± TSG-6 and ± OPG. Osteoclast formation was assessed by TRAP staining and osteoclast activity by lacunar resorption. TSG-6 release in cell culture media by osteoclast precursors and mature osteoclasts was measured using ELISA. TSG-6 expression in synovial tissue was assessed immunohistochemically.

Results: In vitro stimulation of TLR3 and to a significantly lesser extent TLR4, but not TLR2 on RA SF led to strong induction of BAFF (~1,000-fold increase with TLR3) and APRIL mRNA expression. In response to TLR3, BAFF was time-dependently released in the supernatant of RA SF (~300 pg/ml) and, to a lesser extent, OASF. TLR3 stimulation of RA SF in co-culture with B cells strongly enhanced AID expression, ongoing CSR to IgG, but not IgA, as shown by detection of IγCγ1 CT and release of IgG. By contrast, TLR4 stimulation alone had no direct effect on B cells. Conversely, blockade of soluble BAFF/APRIL by BCMA-Ig inhibited TLR3-induced AID-dependent production of AID mRNA, IγCγ1 CT as well as the secretion of IgG.

Conclusions: We demonstrated that RASF are able to release high levels of B cell survival factors upon TLR3 stimulation at both mRNA and protein level. The level of these factors was functional, as demonstrated by the capacity of RA SF to directly modulate AID expression, CSR and production of class-switched antibodies in co-cultured un-switched B cells. This effect was abrogated by blockade of soluble BAFF and APRIL. Overall, these data strongly suggest a dual role for TLR3-1 and TLR4 in cell-cell communication and CSR by RA SF, as measured by APRIL in sustaining functional B cell activation and antibody production.

Disclosure statement: All authors have declared no conflicts of interest.
OP64. OSTEOCLAST-MEDIATED BONE RESORPTION: REGULATION BY HYPOXIA-INDUCIBLE FACTOR (HIF) AND ANGIPOIETIN-LIKE 4 (ANGPTL4)

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Background: Hypoxia is a feature of the hyperplastic synovium in rheumatoid arthritis (RA). Many cellular components of RA express the hypoxia-inducible transcription factor, HIF. We have recently demonstrated that osteoclast-mediated bone resorption is enhanced by hypoxia in a HIF-1α-dependent manner (1). We continue our investigation of the molecular mechanisms regulating hypoxia-induced osteoclast activation to further understanding of bone resorption in RA.

Methods: Osteoclasts were differentiated from CD14+ PBMC with M-CSF (25 ng/ml) and RANKL (50 ng/ml) for 16 days. Osteoclasts were then exposed to hypoxia (2% O2) for 24 h prior to fixation, analysis of resorption (toluidine blue staining of dentine slices) or collection of RNA, protein or cell supernatant. To identify potential genes of interest, an Illumina HumanWG-6 v3.0 48k array was performed comparing 6 paired samples of normoxic vs hypoxic osteoclasts. Transfection of mature osteoclasts with siRNA against HIF-1α or HIF-2α was performed using RNAiMAX (Invitrogen).

Results: Use of a panel of normoxic inducers of HIF (CoCl2, desferrioxamine, dimethyl sulfoxide) or hypoxic osteoclasts, Transfection of mature osteoclasts with siRNA against HIF-1α or HIF-2α was performed using RNAiMAX (Invitrogen). HIF expression is sufficient to enhance osteoclast resorption in the absence of a hypoxic stimulus. Analysis of microarray data therefore focussed on known HIF target genes.

OP65. MOLECULAR AND CELLULAR EVOLUTION OF FUNCTIONAL TERTIARY LYMPHOID STRUCTURES IN SALIVARY GLANDS OF NOD MICE

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Background: Tertiary Lymphoid Structures (TLSs) are common features of chronic inflammatory diseases including Sjögren’s syndrome (SS). We recently showed that these ectopic structures acquire secondary lymphoid organs properties and are capable of supporting B cell activation and autoantibody production including expression of activation-induced cytokine deaminase (AID) and Ig class switching. Dissecting TLSs dynamics in humans is technically and ethically challenging. Thus, we used the NOD mouse, a spontaneous model of autoimmune sialoadenitis, to characterize the cellular and molecular basis of autoreactive B cell activation and evolution of functional Ectopic Lymphoid Structures (ELS) in the chronically inflamed NOD salivary glands.

Methods: Submandibular glands from 110 female NOD mice from 4 to 35 weeks of age were collected. Paired snap-frozen samples were analysed by immunohistochemistry (IHC) for T and B lymphocytes (CD3/CD20) to evaluate cell infiltration and the development of B/ T cell segregation. ELS were detected by staining for FDC-M1 (follicular dendritic cell networks), GL7 (germinal centre B cells) and AID (marker for ELS functionality). Characterization of B cell subsets within the infiltrates was carried out by immunostaining and by FACS analysis with CD19, CD21, CD23, B220, IgD, IgM, CD1d and CXCR5 antibodies. Quantitative TaqMan real-time PCR was performed to investigate the miRNA expression of ELS-related genes. Sex/age matched Balb/c and C57BL/6 mice were used as controls.

Results: NOD infiltrates in glands displayed progressive features of ELS from week 8, with 75% of mice developing B/T cell segregation. FDC networks and GL7+ ectopic germinal centres from week 20. Evolution of TLSs was closely associated with miRNA upregulation of genes regulating ELS organization and function such as lymphoid chemokines CXCL13/CCL19 and their receptors CXCR5/CXCR7, lymphotaxis and B cell survival factors BAFF and APRIL. In agreement with CXCL13/CXCR5 mRNA expression, B cells in infiltrates display strong CXCR5 expression and were mostly characterized by a follicular phenotype (B220−/IgD+/IgM+/CD23+/CD21+). As demonstrated by both IHC and FACS analysis on isolated cells. Finally, functional analysis of ELS was demonstrated by expression of AID mRNA and protein within FDC networks, which paralleled the detection of circulating SS-related autoantibodies.

Conclusions: This work provided the first in-depth characterization of cellular and molecular mechanisms underlying the evolution of functional TLSs within submandibular infiltrates of NOD mice. These data strongly support the hypothesis that B-cells can be activated within TLSs in the target organ and promote in situ autoantibody production. Overall, these data support the critical importance of ELS formation in chronic autoimmune inflammation and identified NOD mice as a suitable model to test therapeutic strategies aimed at modulating B cell functionality.

Disclosure statement: All authors have declared no conflicts of interest.

References

OP66. INDIRECT COSTS ESTIMATION IN PRIMARY SJÖGREN’S SYNDROME

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Background: The aim of this study was to estimate the indirect costs, such as loss of time from work, associated with primary Sjögren’s syndrome (pSS) compared with Rheumatoid Arthritis (RA) and community controls.

Methods: Data were obtained from 34 female patients with pSS as part of a study to develop a systematic activity measure, from 87 consecutive female patients with RA attending a hospital clinic and from 96 female community controls on a general practice list. A modified economic component of the Stanford Health Assessment Questionnaire was used to assess lost productivity. Indirect costs