Conclusion: Imbalances in the numbers and functions of specific lymphocyte cell subsets are key pathogenic derangements in AS, and these insights are leading to changes in clinical practice. The present study provided further evidence on the function and underlying mechanism of lymphocyte subsets, which may be useful in the diagnosis and treatment of ankylosing spondylitis.

REFERENCES

Disclosure of Interests: None declared

AB0036C IMMUNOPHENOTYPIC CHARACTERIZATION OF T-CELL IN PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH GOLIMUMAB
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Background: Golimumab is a human anti-TNF monoclonal antibody that has shown efficacy in RA. In the RA pathophysiology, the elevation of TNF modulates the cellular immune response, which leads to a sustained activation of T-cell response; however, it is unknown whether anti-TNF interferes with the immunophenotype of T-cell.

Objectives: To evaluate activation (CD25, CD69) and exhausted (PD-1, TIM-3, LAG-3, CTLA-4) markers of T-cells in RA patients treated with Golimumab.

Methods: We included 14 patients with RA diagnosis (Criteria ACR/EULAR 2010), with moderate to severe activity; 11 non-responders to synthetic DMARDs (NR-DMARDs) and 3 responders to synthetic DMARDs with pharmacological toxicity (R-DMARDs). All patients were treated with Golimumab during 24 weeks. The questionnaire SF-36, DAS28-CRP, power Doppler signal and expression of CD25, CD69, PD-1, TIM-3, LAG-3 and CTLA-4, in T-cell were evaluated at 0 and 24 weeks.

Results: Clinical variables evaluated in patients with Rheumatoid Arthritis are shown in tables 1 and 2.

The frequency of LAG-3+ on CD4+ T-cells increased after 24 weeks of treatment with Golimumab (p = 0.013) (figure 1). The expression of LAG-3 in CD4+ T-cells (r = -0.586, p = 0.028) (Figure 2) and CD8+ T-cells (r = -0.617, p = 0.019) (Figure 3) inversely correlated with DAS28-CRP after treatment. At the beginning of treatment NR-DMARDs patients showed higher expression of CD25 in CD8+ T-cells and lower expression of TIM-3 in CD4+ and CD8+ T-cells with respect to R-DMARDs. After 24 weeks of treatment, a lower frequency of CD69+ and LAG-3+ T-cells was found and increased of CD25+ T-cells compared to R-DMARDs.

Conclusion: Golimumab treatment increased the expression of LAG-3 in T-cells, suggesting a negative regulator of antigen presentation of T-cells.

REFERENCES

Disclosure of Interests: None declared

Innate immunity in rheumatic diseases

AB0037 NEUTROPHIL GRANULOCYTES ARE PRIMED IN CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS (JIA)
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Background: The adaptive as well as innate immunity is involved in JIA pathology. Neutrophils are key mediators of the innate immune response and is the most abundant cell type found in JIA synovial fluid. Studies of neutrophils in JIA have shown transcriptional abnormalities and neutrophil-derived S100A proteins have shown a potential role as biomarkers. Still, studies of neutrophils in JIA are scarce.
Human neutrophil lipocalin (HNL), also known as neutrophil gelatinase-associ- ated lipocalin (NGAL), is a protein found in the secondary granules of neutrophil granulocytes. HNL can be found in epithelial cells as well, but only in its mono-meric form. The dimeric form is only produced by neutrophil granulocytes. HNL resides in the secondary granules and is released upon stimulation, making HNL a unique marker of primed and activated neutrophil granulocytes, compared to myeloperoxidase (MPO) that resides in the primary granules and is released when neutrophils are activated, but also found in monocytes.

**Objectives:**
To examine levels of the dimeric form of human neutrophil lipocalin (dHNL) to reveal if neutrophil granulocytes are primed in JIA, also analysis of myeloperoxidase (MPO) to reveal neutrophil activation. We aimed to compare the analyses to healthy controls and correlate the results to established measures of disease activity.

**Methods:**
Blood samples from 75 patients with JIA (68% females) and 16 healthy controls were analyzed regarding HNL, using a direct ELISA assay (Diagnostic Development, Uppsala, Sweden) specifically detecting the dimeric form of HNL (dHNL). We also analysed MPO (Diagnostics Development, Uppsala, Sweden), white blood cell count, neutrophil granulocyte cell count, ESR and CRP. All cate- gories of JIA except the systemic category were included. Patient/parent and the physician filled out a global health assessment on a VA-scale (0-10). The number of active joints at sampling was collected and patients were classified according to the ILAR criteria. The participants with JIA had a median age of 12.1 (IQR: 7.7-15.3) years, the control group 5.3 (IQR: 2.0-9.5) years.

**Results:**
The serum levels of dHNL and MPO were significantly elevated com- pared to healthy controls, (p < 0.001; p < 0.002), and correlated significantly with each other (r = -0.68; p < 0.001).

The serum levels of dHNL correlated best to the count of neutrophil granulocytes (r = -0.54; p < 0.001), total leukocyte count (r = -0.43; p < 0.001) and less well to CRP (r = -0.42; p < 0.001) and ESR (r = -0.35; p < 0.002) and not at all to the scoring sys- tem for disease activity, JADAS27 (r = 0.06; p = 0.65).

**Conclusion:**
The increased levels of the dimeric HNL in serum confirmed the involvement of neutrophil granulocytes in JIA although dHNL did not correlate with disease activity. The mechanisms by which neutrophils are primed and acti- vated in JIA, however, still remain an enigma.

**Disclosure of Interests:**
Lillermo Berntsson Consultant for: AbbVie, Speakers bureau: AbbVie, Ulrika Kihlborg: None declared, Per Venge: None declared


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**AB0038**

**S100A9 ASSOCIATES WITH PAIN PERCEPTION IN OA PATIENTS AND INDUCES NOCICEPTIVE PAIN BUT NOT ALLODYNIA IN EXPERIMENTAL SYNOVITIS**

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**Results:**
Acute synovitis was induced by a single i.a. injection of SCW (FAC). Patients with pain at t=5 years after biopsy isolation were compared to those without pain. Inflammation since joint swelling and cell influx were similar in WT and S100A9-/- mice. However S100A9 mice 1 day p.i. WT mice showed a marked and significant decrease in percentage of weight bearing on the SCW injected hindpaw (28%) compared to saline injected mice (P < 0.001) (data not shown). Interestingly, S100A9 mice did not. Gait analysis confirmed that right paws were unloaded in WT mice, not in S100A9 mice. No difference in mechanical allodynia was observed, both mouse strains showed a similar reduction of paw withdrawal threshold (4.2 and 4.5 fold respectively). Analysis of DRG showed no increased phagocyte infiltra- tion after SCW injection as determined by S100A8 and S100A9 IHC and no change in inflammatory gene expression was measured. In addition, no change in F4/80 staining was seen in both WT and S100A9-/- mice. However, expression of neuron activation markers NAV1.7, AT3F and GAP43 was significantly increased at 1 day after SCW injection in WT and not in S100A9-/- mice as compared to saline injected mice (P = 0.022, 0.004 and 0.030, resp.), which is in line with in the reduced pain response observed earlier in S100A9-/- mice. IHC confirmed the dif- ference in NAV1.7 expression in the DRG at protein level.

**Conclusion:**
These findings show that S100A9, which is released from the syno- vium upon inflammation, is an important mediator of inflammatory nociceptive pain response in the knee, rather than by being involved in peripheral sensitiza- tion. In acute inflammation S100A8/A9 is likely regulated via direct activation of TLR4 on nerve endings in the synovium.

**Disclosure of Interests:**
None declared


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**AB0040**

**JAK INHIBITORS – BARICITINIB AND TOFACITINIB – MODULATE THE IN VITRO INFLAMMATORY AND ALTERNATIVE POLARIZATIONS OF MACROPHAGES**

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**Background:**
Macrophages are major effector cells in inflammatory rheumatism such as Rheumatoid arthritis (RA) and Psoriatic arthritis (PsA). Depending on their microenvironnement, especially cytokines content, they can display various pheno- types or “polarizations” described as inflammatory (in the presence of IFNγ or GM-CSF) or as alternative (in the presence of IL-4 or IL-10). These cytokines involve JAK/STAT signaling pathway, and their action is expected to be inhibited by JAK inhibitors (JAKi) developed in RA and PsA. How JAKi modulate polarization of macrophage, and whether this phenomenon explains the clinical benefit in RA and PsA is not fully understood.

**Objectives:**
To evaluate whether JAKi modulate in vitro polarization of mono- cytes derived macrophages.

**Methods:***Cell culture***
CD14+ monocytes were isolated from healthy donors and differentiated as macrophages in the presence of M-CSF. Macrophages were then polarized as inflammatory macrophages by (LPS + IFNγ or GM-CSF), or as alternative macrophages (by IL-4, or IL-10), in the presence or not of JAKi (50 nM): Baricitinib (JAK1/JAK2 specific), Tofacitinib (JAK1/JAK2/JAK3/Tyk2 specific).

**[Membrane markers]**
Surface polarization markers were evaluated by flow cytometry.

**[Functional assays]**
Cytokine production in supernatants of macrophages were quantified by bead-based immunoassay and by ELISA. Reactive oxygen spec- iess (ROS) production triggered by LPS and/or IFNγ was assessed by flow cytometry. Phagocytosis of E.coli bioparticles was assessed by flow cytometry.

**Results:**
We analyzed 12 donors. **Membrane polarization markers:**
CD40/CD80/CD206 for inflammatory macrophages, CD163/CD16/CD206/ CD200R for alternative macrophages. Both JAKi modulated these markers. Regarding the inflammatory polarizations, Baricitinib and Tofacitinib respectively reduced CD40 and CD206 expression. Regarding the alternative polarizations, both drugs inhibited the expression of CD163, CD206 and CD200R.

At the cytokines level, both drugs did not significantly modulate the pro-inflamma- tory/inflammatory-balance in supernatants. We observed a slight increase in TNF and IL-6 resulting from LPS/NFkB pathway [1]. However, Baricitinib decreased the inflammatory IP-10, and increased IL-10 production. Both JAKi did not affect ROS production in the presence of LPS. Consistently with the absence of modulation of CD16 surface expression, Baricitinib and Tofacitinib did not affect CD16-dependent phagocytosis.

**Conclusion:**
Our study shows that despite distinct JAK specificity, Tofacitinib and Baricitinib maintained surface phenotypes close to those of non-activated macrophages This finding was observed whatever was the polarizing condition, thus supporting the potential benefit of JAK inhibitors in different immune diseases.

**REFERENCES**

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