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## Innate immunity in rheumatic diseases

### THU0001 CIRCULATING INNATE LYMPHOID CELLS IN IGG4-RELATED DISEASE

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**Background:** IgG4-related disease (IgG4-RD) is characterized by lymphoplasmacytic infiltrates, $IgG4^+$ plasma cells, fibrosis and frequent ectopic lymphoid structures (ELS). Excessive Th2 cytokines production and role of T follicular helper (Tfh) cells have been reported. Innate cells (ILCs), a heterogeneous population of non-B non-T lymphocytes lacking antigen-specific receptors, are able to produce type 2 cytokines (ILC2s) or to participate to ELS formation (ILC3s) and could contribute to IgG4-RD lesions.
**Objectives:** To analyze circulating blood ILCs in IgG4-RD.
**Methods:** IgG4-RD patients were identified according to the Comprehensive Diagnostic Criteria. Patients treated with steroids or DMARDs within 3 months or rituximab within 6 months prior inclusion were excluded. Peripheral blood mononuclear cells were isolated and analyzed by flow cytometry. ILCs were defined as lymphoid $CD45^+Lineage(Lin)^-CD127^+$ cells. Among ILCs, 3 subsets were defined according to CRTH2 and CD117 expression. In some patients, transcription factor expression (T-bet, GATA3, RORγt) as well as cytokine production (IFNγ, IL-4/IL-13, IL-17/IL-22) after different stimulations were analyzed in ILC subsets. Results were compared to healthy controls (HC), correlated to clinical and biological characteristics, and compared before and after treatment.
**Results:** Twenty patients with active untreated IgG4-RD and 30 HC were included. In IgG4-RD group, mean age was 64 years and sex ratio 4:1. Serum IgG4 levels were >1.35 g/l for 85% of patients and median eosinophil count 410/$mm^3$. Most patients (65%) were analyzed at diagnosis. Main organs involved were lymph nodes (n=15), pancreas (n=9), salivary glands (n=8), biliary tree and kidney (n=5), lung and retroperitoneum (n=4), with ≥3 organs involved in 65%. Number of blood ILCs was not modified in IgG4-RD patients ($1.2 \pm 2.6$ x$10^3$/ml *vs* $1.5 \pm 1.3$ x$10^3$/ml in HC, *p=0.29*; $0.07 \pm 0.06$% of $CD45^+$ lymphocytes *vs* $0.09 \pm 0.05$% in HC, *p=0.35*). ILC1s, ILC2s, and ILC3s represented respectively 30%, 29% and 32% of total ILCs in HC. IgG4-RD patients presented a decrease in ILC2s (23.8% [12.4-29.3], *p=0.04*) and ILC3s (21.2% [8.8-28.1], *p=0.002*) and an increase in ILC1s (54.1% [33.8-68.7], *p=0.0009*) proportions. ILC2s (274/ml [152-594] *vs* 554/ml [62-1000], *p=0.045*) and ILC3s (214/ml [120-620] *vs* 509/ml [336-908], *p=0.04*) numbers were decreased in IgG4-RD patients. No correlation was found between number or proportion of total ILCs or any ILCs subsets and clinical or biological characteristics such as age, number of organ involved, IgG4-RD Responder Index or serum IgG4. No correlation was found between Tfh and ILC3s, or between eosinophils and ILC2s numbers. As human ILC3s and ILC2s can convert *in vitro* into IFNγ producing cells, we explore if ILC plasticity could contribute to ILCs subset distribution observed. GATA3 or RORγT expression by ILC1s was not modified (*p=0.29*, respectively) in IgG4-RD patients compared to HC. As previously reported, no cytokine production (IFNγ, IL-4/IL-13, IL-17/IL-22) was observed after different stimulations of blood ILCs. Analyzes before and after treatment found no difference in proportion or absolute number of total or subsets of circulating ILCs.
**Conclusion:** Circulating ILC2s and ILC3s are decreased in IgG4-RD. Recruitment of ILC2s or ILC3s in tissues, where they could participate to Th2 cytokine production, ELS formation and fibrosis, needs further investigations.
**Disclosure of Interests:** None declared
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### THU0002 THERAPEUTIC ANTI-TNF BIOLOGIC AGENTS EXHIBIT FUNCTIONAL DIFFERENCES IN BLOCKING TNF-INDUCED EFFECTS ON HUMAN MONOCYTES IN VITRO

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**Background:** Therapeutic anti-TNF biologic agents can be distinct in their structure, such as etanercept, a human TNFRII- Fc fusion protein, as compared to adalimumab, a human IgG molecule, and/or in their binding to TNF as shown by crystal structures of adalimumab as compared to infliximab in the presence of TNF. Whether these differences can affect the functional properties of these biologics in direct comparison to each other has not been thoroughly investigated.
**Objectives:** To determine the equivalency of all anti-TNF biologic agents currently approved for the treatment of RA in preventing a variety of TNF-induced effects on human monocytes *in vitro*.
**Methods:** Human monocytic U937 NF-kB luciferase reporter cell line was incubated with 100 ng/mL TNF +/- increasing conc. (0.15-338 nM) of adalimumab (ADA), etanercept (ETN), infliximab (IFX), golimumab (GOL) or certolizumab pegol (CZP) as pre-formed complexes generated for 1 h. at 37°C. Surface TNF-RI and –RII levels were monitored by flow cytometry following 1 h. incubation. Luciferase activity was measured in cell lysate after 4 h. to assess NF-kB activation. After 24 h., U937 cells were analyzed by flow cytometry for surface levels of ICAM-1, an NF-kB-induced adhesion molecule shown to contribute to monocyte migration and arthritis. Apoptosis was assessed by time-lapsed microscopy and flow cytometry using caspase 3/7 fluorescent substrate. Alpha-2,6 sialylation (Sia), a glycosylation modification shown to regulate TNF-RI internalization and apoptosis induction, was evaluated by flow cytometry using FITC-labeled Sambucus nigra lectin (SNA). Human PBMC were incubated with TNF +/- pre-formed complex with anti-TNF biologics for 24 h. and then stained with CD14 and ICAM-1 Abs for flow cytometry.
**Results:** Surface levels of TNF-RI and –RII on U937 cells were both reduced by 2.4-fold in presence of TNF. TNF-RI was maintained at baseline levels by 16.7 nM ADA or CZP as pre-formed complexes with TNF; however, those complexes with ETN, IFX or GOL could only preserve a fraction of this receptor on the surface (43%, 52% and 62%, respectively). All anti-TNF biologics were equally effective in preventing loss of surface TNF-RII. TNF stimulation of U937 NF-kB reporter cells led to a 122-fold increase in luciferase activity which was reduced to baseline by only ADA or CZP with largest conc. range tested. Partial reduction by 3, 7 and 11-fold was observed with ETN, IFX or GOL, respectively. TNF-enhanced ICAM-1 surface expression (3-fold increase) on U937 cells was reduced to baseline by 16.7 nM ADA or CZP, whereas ETN, IFX or GOL were only partially effective (48%, 61% and 59% reduction, respectively). Exposure of CD14+ primary monocytes to ADA:TNF or CZP:TNF complexes not only prevented TNF induction of ICAM-1 but significantly reduced its level below that of baseline, whereas those with GOL or IFX brought ICAM-1 levels to baseline and those with ETN were only 46% effective. Both ADA:TNF and CZP:TNF complexes also completely inhibited TNF-induced apoptosis in a dose dependent manner unlike ETN, IFX and GOL, which were less effective (42%, 32% & 42% reduction, respectively). According to SNA staining, alpha-2,6 Sia surface levels dropped in presence of TNF specifically on the subset of cells undergoing apoptosis, and this subset was reduced proportionately to the inhibitory properties of anti-TNF biologics on apoptosis.
**Conclusion:** For each of the conditions tested *in vitro*, many resembling features associated with RA pathogenesis, the pre-formed complexes of ADA:TNF and CZP:TNF were significantly more effective in preventing the TNF-induced effects (decrease of surface TNF-RI expression and alpha-2,6 Sia; increase in NF-kB activation, ICAM-1 surface expression, and apoptosis) on human monocytes than those complexes of TNF with ETN, IFX or GOL. Additional *in vitro* and *in vivo* studies need to be done to further elucidate the mechanism responsible for these differences.
**Disclosure of Interests:** Bohdan Harvey Shareholder of: AbbVie, Inc., Employee of: AbbVie, Inc., Zehra Kaymakcalan Shareholder of: AbbVie, Inc., Employee of: AbbVie, Inc.
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### THU0003 CRYSTALLINE SILICA IMPAIRS EFFEROCYTOSIS CAPACITIES OF HUMAN MONOCYTE-DERIVED MACROPHAGES THROUGH RHOA-ROCK ACTIVATION

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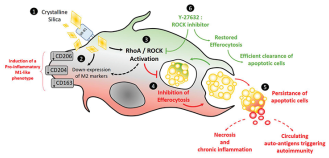
**Background:** Inhalation of crystalline silica can lead to pulmonary diseases and systemic autoimmune disorders, such as systemic sclerosis (SSc), systemic lupus erythematosus or rheumatoid arthritis (1). A failure of apoptotic cell clearance, also called efferocytosis, is reported in autoimmune diseases and, impaired efferocytosis in macrophages from SSc patients has especially been described recently (2). However, the precise

mechanisms linking crystalline silica exposure and autoimmune disorders is still to be determined.
**Objectives:** This study explored the effects of crystalline silica on efferocytosis abilities of human macrophages.
**Methods:** Monocyte-derived macrophages (MDM) were exposed *in vitro* to crystalline silica for 4 hours. Their ability to phagocyte CFSE-positive apoptotic and non-apoptotic Jurkat cells and their polarization profile after silica exposure were assessed by flow cytometry. Efferocytosis capacities of MDM from SSc were also evaluated using the same methods.
**Results:** Crystalline silica exposure impaired efferocytosis capacities of human MDM in a specific and dose-dependent manner. This effect of silica required the expression of SR-B1 and, was associated with a decreased membrane expression of the M2 polarization markers CD206, CD204 and CD163. Their expressions after silica exposure were similar to those of M1 polarized MDM. Silica increased F-actin staining, RhoA activation and phosphorylation of myosin phosphatase subunit 1 (MYPT1), a known ROCK target. Y27632, a Rho kinase (ROCK) inhibitor, reversed the F-actin staining, the phosphorylation of MYPT1 and, at least in part, the silica-induced impairment of efferocytosis. Moreover, efferocytosis abilities of MDM from SSc patients were similar to those of silica-exposed MDM and, a treatment of SSc-MDM with Y27632 significantly increase their efferocytosis capacities, suggesting an activation of the RhoA/ROCK pathway in SSc MDM also.
**Conclusion:** These findings demonstrate that silica impairs efferocytosis in MDM via an activation of RhoA/ROCK pathway **(Figure 1)**. These results also suggest a therapeutic potential of drugs targeting this pathway and advance the hypothesis that silica exposure may contribute to the impaired efferocytosis capacities of macrophages from SSc patients, a silica-associated systemic disorder still without curative treatment to date.




**Abstract THU0003 Figure 1**. Crystalline silica alters polarization of human monocyte-derived macrophages and impairs their efferocytosis capacities through RhoA-ROCK activation

## REFERENCES:

[1] Blanc PD, Järvholm B, Torén K. Prospective risk of rheumatologic disease associated with occupational exposure in a cohort of male construction workers. Am J Med. 2015 Oct;128(10):1094-101.
[2] Ballerie A, Lescoat A, Augagneur Y, Lelong M, Morzadec C, Cazalets C, Jouneau S, Fardel O, Vernhet L, Jégo P, Lecureur V. Efferocytosis capacities of blood monocyte-derived macrophages in systemic sclerosis. Immunol Cell Biol. 2018 Nov 13.

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THU0004

## MICRORNA-223 NEGATIVELY REGULATE GOUTY INFLAMMATION BY TARGETING THE NLRP3 INFLAMMASOME WITHOUT INFLUENCING IL-37 AND TGF-B1

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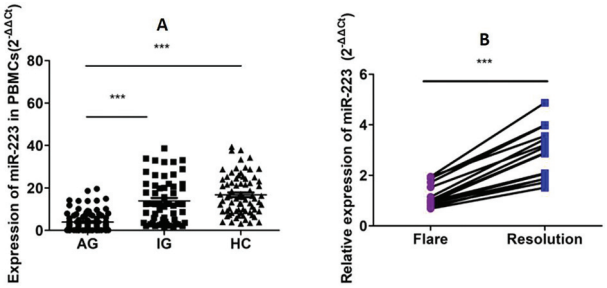
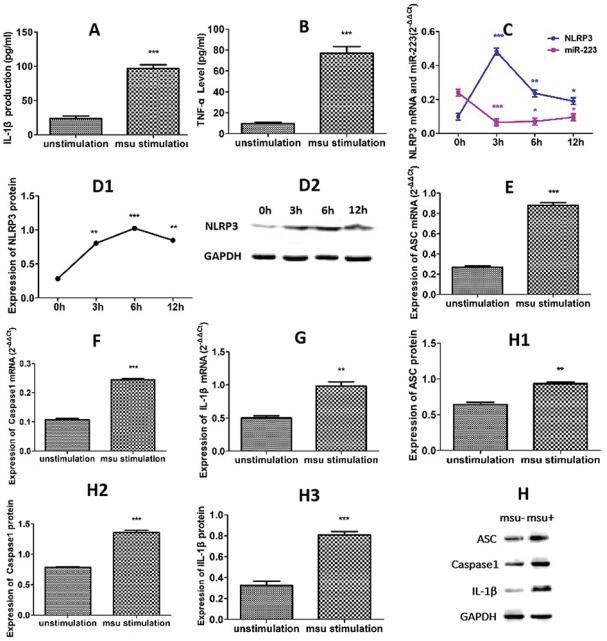
**Background:** MicroRNA-223 (miR-223) serves as an important regulator of inflammatory and immune responses and is implicated in several autoinflammatory disorders[1]. To date, no relevant studies have reported the expression levels of miR-223 in gout patients or assessed whether miR-223 participates in negatively regulating gouty inflammation via regulating cytokines (such as IL-1β, tumor necrosis factor (TNF)-α, IL-37 and TGF-β 1) by targeting the NLRP3 inflammasome.
**Objectives:** To determine the function of miR-223 in monosodium urate (MSU)-induced gouty inflammation.
Methods: miR-223 was detected among 107 acute gout patients (AG), 58 intercritical gout patients (IG), and 75 healthy subjects (HC). RAW264.7 macrophages were cultured and treated with MSU. Over-expression or under-expression of miR-223 was inducted in RAW264.7 macrophages to investigate the function of miR-223. Real-time quantitative PCR, ELISA and western blotting were used to determine the expression levels of miR-223, cytokines and the NLRP3 inflammasome.
**Results:** 1. *Expression of miR-223 in PBMCs among the AG, IG and HC groups(Figure1)*




**Abstract THU0004 Figure 1**.

*2. 2.Altered expression of miR-223, the NLRP3 inflammasome and cytokines in MSU-induced RAW264.7 murine macrophage inflammation (Figure2)*




**Abstract THU0004 Figure 2**.

3. Effect of miR-223 on cytokine secretion from RAW264.7 macrophages treated with MSU(Figure3)