SATURDAY, 15 JUNE 2019

Adaptive immunity (T cells and B cells) in rheumatic diseases

SAT0001

FOSL-2 IS A REPRESSOR OF FOXP3 EXPRESSION DURING TREG DEVELOPMENT AND CONTROLS AUTOIMMUNITY

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Background: Fos like 2 (Fosl-2) is a transcription factor belonging to the AP-1 transcription complex. We have recently described a Fosl-2 transgenic (tg) mouse model which develops a multi-organ inflammatory and autoimmune phenotype. In these mice, we have characterized a decrease in regulatory T cells (Tregs), which preceded the activation of T cells and the development of inflammation.

Objectives: To analyze how Fosl-2 reduces the Treg population and triggers autoimmunity in Fosl-2 tg mice.

Methods: We used previously generated Fosl-2 tg overexpressing and Fosl-2 T cell specific knock out (Fosl-2 ko) mice. For mixed bone marrow transfer experiments, lethally irradiated recipients received a one to one mix of Fosl-2 tg (CD45.2) and wt (CD45.1) bone marrow to create Fosl-2 tg-wt chimera. We addressed the contribution of Tregs to the inflammatory phenotype by co-transferring Fosl-2 tg CD4 T cells and wt Tregs to Rag2^{-/-} recipients. Treg and T cell populations were analyzed by flow cytometry and inflammation was addressed by CD45 staining on paraffin-embedded sections. RNA-sequencing was used to compare wt, Fosl-2 tg and Fosl-2 ko CD4 T cells after 24 hours of stimulation with anti-CD3 (2 µg/ml) and anti-CD28 (2 µg/ml).

Results: We first addressed whether Fosl-2 affected Tregs in a cell intrinsic way using mixed bone marrow experiments. In these animals, the CD45.2 Fosl-2 tg CD4 T cells showed a much lower proportion of Tregs compared to the CD45.1 wt population, both in the spleen (0.73%±0.15 vs 3.16%±3.6, P=0.062) and thymus (1.3%±1.04 vs 3.23%±0.78, P=0.001). This demonstrates that Fosl-2 overexpression represses Treg development in a cell intrinsic way.

In T cell transfer experiments, Rag2^{-/-} mice receiving 10⁶ Fosl-2 tg CD4 cells developed lung inflammation 5 weeks after transfer, confirming that T cells are inducers of inflammation in Fosl-2 tg mice. Moreover, co-transfer of either 3*10⁵ or 10⁶ wt Treg cells resulted in a dose dependent reduction of inflammation. These data indicated that the decrease in the Treg population in Fosl-2 tg mice is responsible for the induction of inflammation.

We then analysed Fosl-2 transcriptional targets in T cells by RNA-seq. Using a fold change > 1.5 and False Discovery Rate (FDR) of 0.05, we identified 191 differentially expressed genes in both Fosl-2 tg and Fosl-2 ko cells compared to wt. Interestingly, one of the top target genes of Fosl-2 was FoxP3. This unbiased approach thus revealed that FoxP3 expression is repressed by Fosl-2, with a 6.5 fold reduction in Fosl-2 tg cells and a 2.5 fold increase in Fosl-2 ko cells. This effect was confirmed on the protein level with a reduction in FoxP3 induction in Fosl-2 tg cells treated with TGFβ. The repression of Treg development observed in Fosl-2 tg mice could thus be explained by a direct transcriptional control of FoxP3 expression. Additionally, we found that Fosl-2 repressed a set of genes important for Tregs and other T helper cells. This included Nr4a2, a transcription factor involved in Treg development, IRF8 and Eomes, two genes involved in the polarization of Th1 and Th17 cells, or Ccl1 a chemokine important for Treg homeostasis.

Conclusion: Fosl-2 is involved in the control of FoxP3 expression in T cells. Through this, overexpression of Fosl-2 represses Treg development and induces a Treg dependent autoimmune phenotype in mice. This mechanism could thus be involved in the pathogenesis of autoimmune diseases and might represent a therapeutic target to modulate the Treg population.

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Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, AnaMar, Bayer, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/has consultancy relationship within the last 3 years with A. Menarini, Amgen, Abbvie, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritides and related disorders

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SAT0002

DIFFERENTIAL RECONSTITUTION OF B-CELL SUBSETS IN SYSTEMIC SCLEROSIS PATIENTS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Autologous hematopoietic stem cell transplantation (AH SCT) is nowadays a clinical reality as an effective and safe approach to treat systemic sclerosis patients' refractory to conventional therapy using immune suppressive drugs, although its immune mechanisms are still not completely understood^{1,2,3}.

Objectives: To evaluate the reconstitution of B-cell subsets in SSc patients following AH SCT.

Methods: Peripheral blood samples were harvested from twenty-four SSc patients before transplantation and at 30, 60, 120, 180 and 360 days post-AH SCT. The immunophenotyping, regulatory B-cell IL-10 production and suppressive assays were assessed by flow cytometry.

Results: Compared to baseline, naïve B-cells (CD19⁺CD27⁺IgD⁻) significantly decreased in frequency and absolute counts at 30 days post-AH SCT, followed by an increase at 360 days. There was a transient decrease of non-class-switched memory-B-cell (CD19⁺CD27⁺IgD⁺) frequency at 30 days, followed by an increase at 360 days. In addition, mature class-switched memory-B-cells (CD19⁺CD27⁺IgD⁺) and plasma cell (CD19⁺CD27^{hi}IgD⁺) decreased (P<0.05) respectively at 60 and 360 days post-AH SCT, while the frequency of double-negative B-cell (CD19⁺CD27⁺IgD⁻) increased at 30 days post-AH SCT. The subset CD20⁺CD43⁺CD27⁺CD69⁻ (human B1-cell) showed higher percentage at 30 days, followed by a decrease at 360 days. The regulatory B cell CD19⁺CD24^{hi}CD38^{hi} (transitional B-cell subset) significantly increased at 360 days, while CD19⁺CD24^{hi}CD27⁺ Breg (B10 subset) decrease transiently from 30 to 180 days post-AH SCT. The frequency of CD19⁺PD1⁺ B-cells transiently increased at 30 days. The frequency of IL-10-producing regulatory B-cells CD19⁺CD24^{hi}CD38^{hi} and CD19⁺CD24^{hi}CD27⁺ increased post-transplantation. After AH SCT the regulatory B-cell CD19⁺CD24^{hi}CD38^{hi} recover their ability to suppress cytokine production by Th1 CD4⁺ T-cell in vitro.

Conclusion: Following transplantation, SSc patients displayed increased naïve-B-cells values and decreased memory B-cells, which might contribute to self-tolerance reestablishment, disease remission and clinical improvement on these patients. SSc patients showed increases of Breg numbers after AH SCT as well as an increase of IL-10 production, suggesting improvements in immunoregulatory mechanisms.

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SAT0003

PIM1 REGULATES CD4+ T CELL EFFECTOR FUNCTION IN EARLY RHEUMATOID ARTHRITIS AND HAS POTENTIAL FOR USE AS A NOVEL, MEASURABLE THERAPEUTIC TARGET

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Background: We previously showed PIM1, among other STAT3-target genes, to be strikingly upregulated in circulating CD4+ T cells of treatment-naïve 'early' rheumatoid arthritis (eRA) patients^{1,2}. PIM1 has a recognised role in T cell development, has been implicated in the pathogenesis of autoimmunity³, and is the target of specific inhibitors in clinical development in oncology; for example PIM1 was recently identified as a promising target in triple-negative breast tumours⁴.

Objectives: We sought to understand the relevance of dysregulated PIM1 gene transcription for T-cell pathobiology during disease initiation and to determine the effects of its inhibition on T cell function. We hypothesised that, amongst a readily-identifiable, high PIM1 expressing subgroup of eRA patients, PIM1 overexpression in CD4+ T cells is a targetable early event in pathogenesis that lies downstream of STAT3-mediated IL-6 signalling.

Methods: Peripheral blood was obtained from consenting treatment-naïve arthritis patients or healthy donors and highly pure CD4+ T cells were isolated. PIM1 knock-down (SMARTpool siRNA, Dharmacon) or protein inhibition (small molecule inhibitors; PIM1-selective TCS-PIM-1 1, Tocris and panPIM AZD1208) was undertaken. Flow cytometric analysis was then used to assess activation and proliferation following CD3/CD28-mediated stimulation.

Results: In eRA, *ex vivo* CD4+ T cells exhibited an activated, hyper-proliferative phenotype compared with those isolated from healthy donors. Significantly higher PIM1 mRNA expression in CD4+ T cells was seen using PrimeFlow (flow cytometry) compared to that of other inflammatory arthritides. Both PIM1 knock-down, PIM1-specific and panPIM inhibition decreased the activation and proliferation of stimulated eRA (Figure 1A) and healthy donor CD4+ T cells. Moreover, in eRA, PIM1 and panPIM inhibition increased FoxP3 expression (Figure 1B) and increased the proportion of regulatory T cells (Figure 1C).

Figure 1. Early RA CD4+ T cell (A) proliferation is significantly decreased by either a PIM1-selective or a panPIM small molecule inhibitor, whereas (B) FoxP3 expression and (C) the proportion of regulatory T cells is increased.

Conclusion: Taken together, these data implicate PIM1 as prominent among genes whose induction may "pre-programme" CD4+ T cells to function aberrantly in disease. Conceivably, targeting PIM1 may prove an attractive approach to regulating aberrant CD4+ T cell effector function in an identifiable sub-population of early RA patients that exhibit high CD4+ T cell PIM1 expression in their peripheral blood.

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SAT0004

EVALUATION OF AUTOPHAGY IN INFILTRATING AND CIRCULATING LYMPHOCYTES FROM PATIENTS WITH SJOGREN'S SYNDROME

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Background: Autophagy is a lysosome mediated catabolic process that promotes cell survival during stress conditions. Aberrant regulation of autophagy is involved in the pathogenesis of a widening spectrum of autoimmune disorders: in particular, activation of autophagy pathway is implicated in aberrant survival of activated T cells. We recently demonstrated that autophagy is up-regulated in CD4+ T lymphocytes from patients with Sjogren's Syndrome (SS) both in peripheral blood and tissue. Moreover, aberrant autophagy correlates with systemic disease activity. Little is known on the role of autophagy in B cells homeostasis in SS.

Objectives: To investigate the activation of the autophagy pathway in lymphocytes infiltrating salivary gland in SS and peripheral blood from patients with SS.

Methods: Frozen SS minor salivary glands (MSG) from 20 patients were sectioned and evaluated with immunohistochemistry (IHC) by staining for CD3+, CD20+, and CD21+. MSG sections, sequential to IHC, were stained by Cresyl Violet and microdissected (Laser Capture Microdissection) in order to isolate and collect small infiltrates, large CD21- infiltrates, and large CD21+ infiltrates (GC-like structures). Microdissected GC from frozen human tonsils were used as control. The expression of autophagy genes Atg5 and MAP1LC3II (expressed as 2^{-deltaCT} normalized to GADPH) was determined by qPCR on microdissected tissue.

For studies on peripheral blood, 19 SS patients and 11 healthy controls were enrolled, and autophagy, expressed as the ratio between Mean Fluorescence Intensity and the isotopic control (rMFI), was evaluated by Cyto-ID[®] Autophagy detection kit in T lymphocytes (CD4+ and CD8+) and B lymphocytes (CD19+) using a FACSCalibur cytometer.

Results: Expression of the autophagy genes Atg5 and MAP1LC3 was significantly higher in the GCs from SS salivary glands compared to control tonsils (p<0.0001 and p=0.0013, respectively). In the salivary glands of SS patients, progressively higher expression levels of both Atg5 and MAP1LC3 mirrored the organization and severity of the inflammatory infiltrates (small vs. large CD21+ infiltrates p=0.0005 and p<0.0001 respectively).

Immunophenotyping studies revealed activation of the autophagy pathway in both circulating CD19+ and CD4+ lymphocytes from SS (p=0.04 and p=0.03 compared to HC); conversely, no pathway activation was detected in CD8+ cells. Of note, in patients with SS activation of autophagy was most marked in circulating CD19+ B cells compared the other subsets [(CD19+ vs CD4+ (p<0.0001); CD19+ vs CD8+ (p= 0.001)].

Conclusion: Our findings revealed that the autophagy pathway is aberrantly activated in lymphocytes infiltrating salivary glands of SS patients. Of note, progressively higher expression levels of autophagy genes mirrored the severity and extension of inflammatory infiltrates. Immunophenotyping studies revealed that activation of autophagy is preminent in B cells. Thus, activation of autophagy emerges as a feature of both infiltrating and circulating SS lymphocytes, and as mechanism possibly implicated in the activation and survival of autoreactive cells.

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