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, anti-CD3 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 31.6%±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.

Disclosure of Interests: Florian Renoux: None declared, Mara Stellato: None declared, Alexander Vogetseder: None declared, Riyung Huang: None declared, Arun Subramaniam: None declared, Przemyslaw Blyszczuk: None declared, Jörg Distler: None declared, Gabriela Kania: None declared, Onur Boyman: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer,

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/had 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

DOI: 10.1136/annrheumdis-2019-eular.2841

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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