Conclusion: Combined, our findings suggest that the CD4⁺CD161⁺CCR2⁺CCR5⁺ T cell subset represents a substantially abnormal T cell subset in RA, exhibiting exaggerated pro-inflammatory responses, numerical abundance relative to Tregs, and resistant to regulation by Tregs. The CD4⁺CD161⁺CCR2⁺CCR5⁺ T cell subset appears to be a marker of therapeutic response status in RA, via its contribution to disease pathology and highlights this subset as a potential therapeutic target in RA.

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THU0040

PROTEINASE 3-REACTIVE B CELL RECONSTITUTION AFTER TREATMENT WITH RITUXIMAB FOR ANCA-ASSOCIATED VASCULITIS

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Background: Proteinase 3 (PR3)-reactive B cells are present in PR3-ANCA-associated vasculitis (AAV) at levels higher than healthy controls.

Objectives: To evaluate the dynamics of the PR3-reactive B cell repopulation in patients with PR3-AAV after treatment with rituximab, and to analyze possible associations between these immunological changes and long-lasting remissions.

Methods: We analyzed all available frozen peripheral blood mononuclear cells (n=148) from 23 randomly-selected PR3-AAV patients who participated in the RAVE trial and achieved complete remission (BVAS=0, prednisone=0) after treatment with rituximab.

We measured PR3-reactive B cells and the relative subsets by a multi-color flow cytometry panel including CD19, IgD, CD27, CD38, CD24, and a biotinylated PR3 revealed by fluorescent streptavidin. The clinical data of the trial were correlated with flow-cytometry data.

Results: 10/23 (43%) patients relapsed during the follow up, 8/10 relapses were severe. At baseline, clinical features, PR3-ANCA levels, % of total PR3-reactive B cells and PR3-reactive B cell subsets were similar between relapsers and non-relapsers. All patients were followed until the end of the trial, for a mean of 44 months (25-75%IQR 31-54), without difference in follow-up time between relapsers and non-relapsers (p=0.98).

The majority of patients had B cell repopulation at 12 (range 12-24) months after rituximab. At the time of B cell repopulation, transitional (CD19+CD24+CD38+) and naïve (CD19+CD27+IgD-) B cells were higher compared to baseline, while total plasmablasts (PB) were unchanged, and mature B cells significantly decreased in both relapsers and non-relapsers. PR3-reactive B cells reappeared in all the patients, and the % of PR3-reactive of B cells were higher at the B cell repopulation visit compared to baseline (5.82% vs 4.25%, p<0.05), while total B cells were lower (66/μL vs 151/μL, p<0.01), regardless of future relapse.

Within PR3-reactive B cells, only the % of PB (CD19+CD27+CD38+PR3+) were higher in relapsers vs. non-relapsers (median [25-75%IQR]; 1.95% [1.315-3.845] vs 0.84% [0.05-1.66], p=0.022) and severe relapsers vs non-severe relapsers (2.165% [1.66-4.315] vs 0.84% [0.1-1.74], p=0.015). Time-to-relapse and time-to-severe-relapse were significantly shorter in patients with circulating PR3-PB higher than the median value of the cohort (1.6%) during B cell reconstitution (Figure 1A-B).

Conclusion: In PR3-AAV, during B cell reconstitution after rituximab, the total fraction of PR3-B cells increases, due to the expansion of the transitional and naïve B cell compartments. Circulating PR3-PB within PR3-B cells are enriched in the peripheral blood of relapsing and severely relapsing patients compared to non-relapsing patients. Higher levels of PR3-PB after rituximab during B cell reconstitution significantly increased the risk of subsequent relapse and severe relapse.

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THU0041

IFNA AND IL21 PROMOTE DISTINCT POPULATIONS OF EFFECTOR B CELLS

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Background: B cells play a crucial role in the pathogenesis of systemic autoimmunity through various effector functions, including auto-antibody production, secretion of pro-inflammatory cytokines and antigen presentation to T cells. Interferon alpha (IFNα), mainly produced by innate cells (Menon et al., 2016), and interleukin (IL)-21 which is secreted by follicular helper T cells (Berglund et al., 2013), promote the generation of auto-reactive IgG-secreting plasma cells. However, it is suggested that IFNα participate also to the generation of regulatory B cells.

Objectives: To further understand the disturbing microenvironmental signals leading to autoimmune diseases, we aim to define a coherent framework integrating the B-cell subsets having different ability to respond to microenvironmental signals, and the signalling pathways driving the differentiation and the functional fate of B cells.

Methods: Naïve and several populations of memory B cells were isolated from peripheral blood of healthy donors and differentiated *in vitro* in the presence of IL21 or IFNα. The phenotype and the expression of transcription factors were analysed by flow cytometry and molecular identity of these cells was further determined by transcriptomic approaches. Functional analyses were performed to assess the effector functions of B cells and their potential regulatory effects on T cells.

Results: IFNα, in synergy with CpG, promote the generation of CD27^{high} CD38^{high} plasmablasts (PB), mainly arising from memory subsets, but not IL21. However, IFNα and IL21 induce the transcriptional program of B-cell differentiation by up-regulating IRF4 and Blimp1 expression. Unlike IFNα, IL21 drive the expansion of CD11c⁺ T-bet⁺ cells only from switched memory subsets, suggesting T-bet may be a footprint of long-lived memory B cells. Even though subtle differences were observed in the antibody production between IFNα and IL21-stimulated memory B cells, naïve cells secreted less amount of IgM and IL10 when stimulated with IL21.

Transcriptomic studies are still in progress to further define the molecular profile of those distinct effector B cells.

Conclusion: Taken together, these findings suggest that IFNα promote a rapid differentiation of B cells into IL10 and Ig-secreting PB whereas IL21 contributes to the generation of T-bet⁺ atypical pre-PB that may have a role in chronic autoimmune disorders.