SAT0012

DETECTION OF CIRCULATING PR3-SPECIFIC B CELLS IN PATIENTS WITH ACTIVE ANCA-ASSOCIATED VASCULITIS

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Background: We recently reported that, in a small number of subjects, the proportion of proteinase 3 (PR3)-specific B cells was higher in patients with PR3-ANCA-associated vasculitis (AAV) compared to healthy controls (HCs).

Objectives: The objectives of this work were to replicate our previous findings on a larger number of subjects, including myeloperoxidase (MPO)-AAV patients as disease controls, and to study the association of PR3-specific B cell proportions with clinical and biological features in patients with PR3-AAV.

Methods: We analyzed frozen PBMCs from 166 patients who participated in the RAVE trial, including 113 patients with PR3-AAV and 53 patients with MPO-AAV, as well as 27 HCs. We measured the proportion of PR3-specific B cells and subsets among PBMCs using a multi-color flow cytometry panel including CD19, IgD, CD27, CD38, CD24, and a biotinylated PR3 revealed by fluorescent streptavidin. In parallel, one million PBMCs from each sample were cultivated in vitro for 12 days and stimulated by CpG, BAFF, and IL-21. PR3-ANCA levels were quantified in the culture supernatants and in the serum.

Results: The mean (SD) proportion of PR3-specific B cells was higher in patients with PR3-AAV compared to patients with MPO-AAV and to HCs: 2.78% (2.64) vs 1.97% (1.2) vs 1.24% (0.49) respectively, $p < 0.001$ for each comparison. We observed a shift towards a more mature phenotype of these PR3-specific B cells in patients with PR3-AAV compared to MPO-AAV and HCs, as represented by a higher proportion of PR3-specific B cells among the switched memory (IgD neg, CD27 pos) B-cell subset, respectively 2.20% (1.99) vs 1.3% (0.72) vs 0.71% (0.40), $p < 0.001$. In patients with PR3-AAV, we did not observe any associations between PR3-specific B cell proportion and age, sex, new diagnosis vs relapsing disease, or disease activity (BVAS/WG). Serum PR3-ANCA levels were significantly correlated with in-vitro PR3-ANCA secretion (Spearman $r = 0.44$, $p < 0.001$). The ratio of PR3-specific B cells in the switched memory/naïve B cell subsets showed a low correlation with serum PR3-ANCA levels ($r = 0.20$, $p = 0.04$), but not with in-vitro PR3-ANCA secretion.

Conclusion: The proportion of circulating PR3-specific B cells is higher in patients with PR3-AAV than in patients with MPO-AAV and HCs, and importantly, their repartition is shifted towards a memory phenotype. Further studies are ongoing to characterize the dynamics of the PR3-specific B cells after therapy, and to understand the differences of these autoreactive B cells between patients with PR3-AAV and HCs.

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SAT0013

CHANGES AND SIGNIFICANCE OF CD4+CD25+FOXP3+ TREG CELLS IN THE PERIPHERAL BLOOD OF PATIENTS WITH AUTOIMMUNE DISEASES

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Background: In recent years, studies have found that the maintenance of immune tolerance of Treg cells plays a pivotal role in the occurrence and development of AID, and that the mutual regulation and dynamic equilibrium between Treg cell and other T lymphocyte plays a significant role in maintaining the immune homeostasis within the body. Therefore, the present research proposes to explore the status of the absolute numbers of Treg cells among various AID patients and correlation with disease activity using a large sample.

Objectives: To determine CD4⁺CD25⁺Foxp3⁺T regulatory (Treg) cell levels in peripheral blood (PB) of patients with autoimmune diseases (AID) and age- and sex-matched healthy controls, and to explore the correlation between the levels of peripheral Treg cells and clinical parameters in AID.

Methods: A total of 1561 AID patients, and 196 age- and sex-matched healthy controls were enrolled. The absolute numbers of CD4⁺CD25⁺Foxp3⁺Treg and other T subsets [total T, CD4⁺T, CD8⁺T, T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17) cells] in PB were measured by Flow Cytometer (FCM). Erythrocyte sedimentation rate (ESR) was analyzed by the Westergren method. Serum concentrations of C-reactive protein (CRP) were measured by monoclonal immunoassay.

Results: (1) The absolute numbers of Treg cells in PB of patients with AID [22.90 (18.31, 36.47)] were significantly lower than those of healthy controls [30.24 (21.85, 41.34); $p < 0.05$], which was also seen in rheumatoid arthritis (RA), Sjogren's syndrome (SS), systemic lupus erythematosus (SLE), systemic vasculitis (SV), idiopathic inflammatory myopathy (IIM) and other diseases. The levels of CD4⁺T, Th1/Treg, and Th2/Treg in PB of AID patients were higher than those of healthy controls. (2) The absolute numbers of most T cell subsets in PB of most AIDs were lower than those of healthy controls, but the Treg cells were greatly decreased, so the ratio of Treg to other T lymphocyte subsets were higher than those of healthy controls. (3) The levels of inflammatory indicators were inversely associated with numbers of Tregs, total T, CD4⁺T, CD8⁺T, Th1 and Th1/Treg cells.

Conclusion: In AID, the absolute numbers or function of CD4⁺CD25⁺Foxp3⁺Treg cells were decreased, so it cannot effectively maintain immune tolerance or inhibit inflammatory response, which may be one of the important mechanisms of disease. Treatment promoting CD4⁺CD25⁺Foxp3⁺Treg cell function may become a new strategy for AID therapy.

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