AB0029  CITRULLINE REACTIVE B CELLS ARE PRESENT IN THE LUNGS OF EARLY UNTREATED RA

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Background: We have previously shown that structural changes, increased tissue citrullination, signs of local inflammation and ACAP are present in the pulmonary compartment of early seropositive RA. These findings suggest a potential role for the lungs in generation of RA-associated autoimmunity.

Objectives: To identify citrulline reactive B cells in the lung compartment of early untreated RA patients and to generate and characterize the corresponding monoclonal antibodies.

Methods: Bronchoalveolar lavage (BAL) fluid cells (13 and 22.5 million respectively) were obtained from two early untreated non-smoking ACPA positive RA patients and single CD19+ B cells were sorted by flow cytometry. Immunoglobulin variable region genes were sequenced and expressed to generate recombinant monoclonal antibodies (mAbs). The citrulline reactivity was determined by in-house ELISA against different citrullinated peptides and controls.

Results: Single sorted CD19+ B cells (n=768) from each patient (RA.1 and RA.2) were processed and the variable region amplification and sequencing yielded 192 pairs of heavy chain sequences from each patient were ACAPs as determined by their reactivity against CCP2. The 4 ACAPs have varying ACPA fine specificity against citrullinated enolase, filaggrin, vimentin and fibrinogen peptides (figure 1). Sequence analysis of the heavy chain variable region revealed unique V gene usage of the ACAPs arising from different patients, V4-39 for the 2 mAbs from RA.1 and V3-49 for RA.2.

Conclusion: We demonstrate for the first time that citrulline-reactive B cells are present in the lung compartment of early untreated RA patients and that citrulline-reactive B cells can be isolated for further characterization.

REFERENCE


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AB0030  QUANTIFICATION OF CD27+ MEMORY B-CELLS IN RHEUMATOID ARTHRITIS PATIENTS TREATED WITH RITUXIMAB

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Background: Rituximab (RTX) is being increasingly used in treatment of several autoimmune diseases, including Rheumatoid Arthritis (RA). RTX induces a deep depletion of all peripheral B-Cell subsets (memory and naïve B-cells). During the B-Cell repopulation phase, occurring approximately after 3 months of RTX administration, B-precursors and naïve cells reappear. Several studies have shown that relapsing RA patients are characterized by a relative expansion of memory B cells during the B-cell repopulation phase.

Objectives: The aim of this study was to quantify the memory B-cell compartment in RA patients with different disease activity scores, evaluated by DAS28, during RTX treatment.

Methods: 26 RA patients under RTX treatment were studied. At the end of the treatment, 26 showed high-to-moderate activity risk (median DAS28=4.8) and 18 low activity risk or remission (median DAS28=2.69). After a median of 3 months from last RTX infusion, B-Cell subsets (precursors, naïve, memory B cells and plasma cells) were quantified in peripheral blood by flow cytometry, using a panel of 8 markers (CD3, CD4, CD6, CD19, CD20, CD27, CD38 and CD45). B naive cells were identified as CD19+ CD20+ CD27-; B memory cells were identified as CD19+ CD20+ CD27+; B plasma cells as CD19+ CD38++ CD27+ and B precursors as CD19+ CD38+ CD20- CD27-. Percent and absolute values were calculated for each subset. In addition, 10 healthy subjects were included as negative control group (NC).

Results: The median percent and absolute values of B naïve cells, B memory cells and plasma cells identified in NC, non-responders RA patients with high/moderate disease activity and responder RA patients with low disease activity or in remission are reported in Table 1.

The virtual absence of peripheral B-Cell was defined as <0.1 B-Cell/µL. In the responder group, 5/18 cases showed absolute B-Cell levels >0.1 cell/µL, while only in 1/8 of the non-responder group a similar B-Cell depletion was found. The memory B-Cell% was significantly higher in non-responders than in responders (p<0.05); the memory B-Cell level in non-responders was similar to that of the NC group.

Conclusion: We used a sensitive and easily applicable flow cytometric multicolor panel that allowed the accurate and standardized identification and enumeration of peripheral blood B-Cell subsets. As reported by other studies, higher levels of memory B-Cells were found in non-responder RA patients treated by RTX, approaching those of healthy individuals.

REFERENCES


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