to a healthy control group (7.6% versus 5.85%, $p = 0.129$). After a 12-week treatment with alfacalcidol in patients with RA, an increase in the percentage of activated Treg cells (HLA-DR + relative to total T-reg lymphocytes) was observed in relation to the values detected at the beginning of the study, up to the level recorded in the group of healthy controls 6.13% vs. 4.76%, $p = 0.219$).

Conclusion: It is believed that functional blockade of Treg cells plays the most important role in RA immunopathogenesis, most likely due to inhibition of their function by proinflammatory cytokines, due to an increase in the number of activated effector T cells, or perhaps due to the fact that some completely differentiated T reg cells can be very unstable. Our results are in favor of the potential immunomodulatory effect of alfacalcidol in autoimmune Th1/Th17-mediated diseases.

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SAT0029

THE IMMUNE-PATHOGENIC CHARACTERISTICS OF AUTOACTIVE B CELLS AGAINST CITRULLINATED ANTIGENS IN RHEUMATOID ARTHRITIS

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Background: Autoreactive B cells are critical mediators of autoimmune pathology. At present, little is known about the functional and phenotypic characteristics of these cells in human autoimmune disease. Rheumatoid arthritis (RA), a common autoimmune disease, is characterized by the presence of disease-specific anti-citrullinated protein antibodies (ACPA). Different lines of evidence indicate that CD20+ B cells, and in particular the citrullinated antigen-specific, ACPA-expressing subset, are critically involved in disease pathogenesis.

Objectives: To delineate the molecular make-up of ACPA-expressing B cells and to define their pathogenic effector functions.

Methods: Protective tetanus toxoid (TT)-specific and autoreactive ACPA-expressing B cells were identified and analyzed directly ex-vivo from individual RA patients and from ACPA-positive individuals at-risk for developing disease. Both antigen-specific cell populations were enumerated and phenotypically characterized by flow cytometry. In addition, ACPA-expressing B cells were isolated and functionally analyzed in B cell culture systems.

Results: In contrast to TT-specific B cells from the same patients, ACPA-expressing B cells were larger in size and strongly expressed CD19, HLA-DR, CD80, CD86 and the proliferation marker Ki-67, while down-regulating CD32. This activated phenotype was less pronounced in ACPA-positive 'at-risk' arthralgia patients. Furthermore, ACPA-expressing B cells in blood of RA patients secreted elevated levels of pro-inflammatory cytokines, in particular IL-8, upon stimulation and did so spontaneously as differentiated plasmablasts/cells in synovial fluid. Notably, ACPA-expressing B cell-derived IL-8 induced neutrophil migration in vitro.

Conclusion: Our findings provide first evidence that ACPA-expressing B cells are well equipped to activate T cells, actively differentiate into IL-8 producing plasmablasts and, hence, could attract neutrophils to the rheumatoid joint. These findings define important phenotypic and functional characteristics of autoreactive B cells in a prototypic human autoimmune disease. They point to a direct pathogenic role of ACPA-expressing B cells in the inflammatory disease process underlying RA and favour approaches that aim at their antigen-specific depletion.

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SAT0030

CITRULLINE-REACTIVE B CELLS ARE PRESENT IN INFLAMED GINGIVAL TISSUE AND DISPLAY CROSS-REACTIVITY BETWEEN BACTERIAL AND HUMAN ANTIGENS

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Background: Antibodies targeting citrullinated proteins (ACPA) are highly specific for rheumatoid arthritis (RA). However, the etiology and molecular basis for ACPA production is still unclear. Based on an epidemiological association between RA and periodontitis (PD), and the unique ability of the oral pathogen *P.gingivalis* (Pg) to express a PAD enzyme that can citrullinate both bacterial and human proteins, it has been hypothesised that the ACPA response may be triggered in the gum mucosa in response to Pg.

Objectives: The main purpose of this study was to investigate if citrulline-reactive B cells reside in inflamed gingival tissue, and to examine ACPA cross-reactivity between citrullinated bacterial and human epitopes on a monoclonal level.

Methods: Using a single-cell antibody cloning approach, 55 recombinant monoclonal antibodies (mAbs) were generated from gingival tissue (GT) CD19+ B cells (n=1 ACPA+ RA/PD patient). Citrulline reactivity was determined using the anti-CCP2 kit (EuroDiagnostica AB), and in-house peptide ELISAs (including a citrullinated peptide derived from Pg PAD (CPP3) and citrullinated peptides derived from human α -enolase, fibrinogen, vimentin, filaggrin and histone 4). Reactivity against CCP2 and CPP3 was also investigated in: 19 mAbs from bronchoalveolar lavage (BAL) CD19+ B cells (n=2 ACPA+ RA patients); 29 mAbs from bone marrow (BM) plasma cells (n=4 ACPA+ RA patients); 142 mAbs from synovial fluid (SF) plasma cells (n=5 ACPA+ RA patients); and 36 mAbs from peripheral blood memory/plasma cells (n=4 ACPA+ RA patients). Predicted germline versions were produced for two of the mAbs.

Results: Among 55 GT mAbs, 14 unique clones (25%) were reactive to the bacterial CPP3-peptide. We also detected CPP3-reactive mAbs from BAL (n=9/19), BM (n=3/29), SF (n=1/142) and peripheral blood (n=1/36). Interestingly, 11 out of 28 (39%) CPP3-reactive clones also bound citrullinated peptides derived from human proteins. Notably, three of these clones were positive in the clinical anti-CCP2 test, and when converted back to the predicted germline sequence, these clones became CCP2 negative, while maintaining reactivity against the bacterial CPP3 peptide.

Conclusion: Our data show that B cells reactive with a citrullinated peptide derived from Pg PAD are present in gingival tissue, lungs, bone marrow, blood and the inflamed joint of ACPA+ RA patients. Moreover, the finding that a number of these clones are cross-reactive with citrullinated peptides derived from human proteins as well as the gold standard CCP2 test suggests mechanisms of molecular mimicry in the generation of ACPA. Importantly, the germline versions of these ACPA were Pg-reactive but not autoreactive, supporting the hypothesis of a bacterial origin for this ACPA response.

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