Dysregulation of proinflammatory versus anti-inflammatory human T_H17 cell functionalities in the autoinflammatory Schnitzler syndrome

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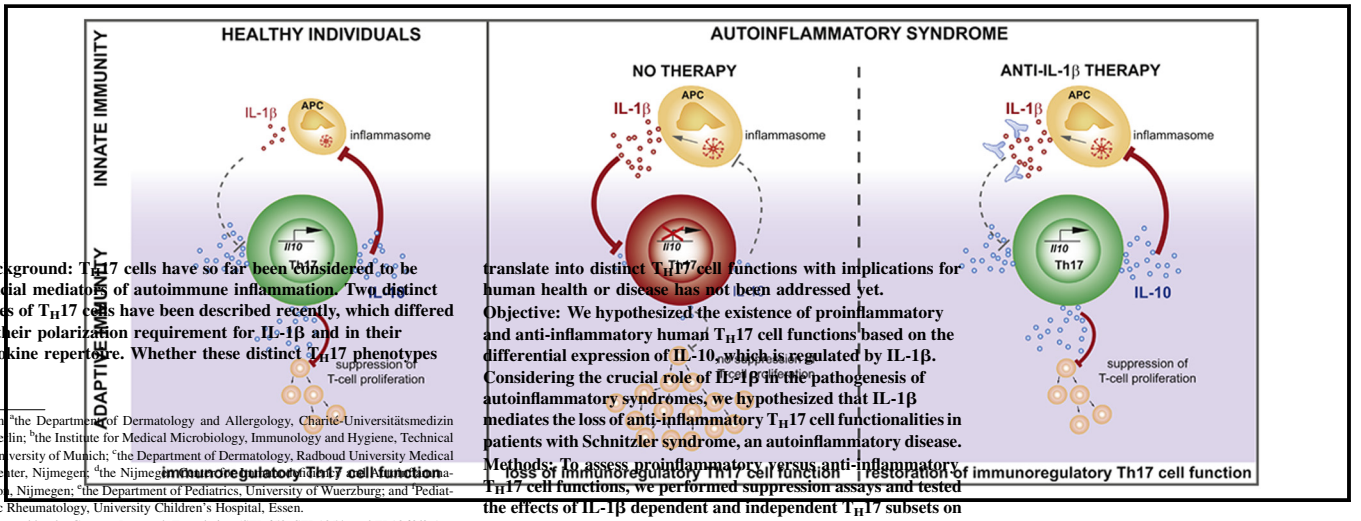
GRAPHICAL ABSTRACT



Background: T_H17 cells have so far been considered to be crucial mediators of autoimmune inflammation. Two distinct types of T_H17 cells have been described recently, which differed in their polarization requirement for IL-1β and in their cytokine repertoire. Whether these distinct T_H17 phenotypes translate into distinct T_H17 cell functions with implications for human health or disease has not been addressed yet.

Objective: We hypothesized the existence of proinflammatory and anti-inflammatory human T_H17 cell functions based on the differential expression of IL-10, which is regulated by IL-1β. Considering the crucial role of IL-1β in the pathogenesis of autoinflammatory syndromes, we hypothesized that IL-1β mediates the loss of anti-inflammatory T_H17 cell functionalities in patients with Schnitzler syndrome, an autoinflammatory disease.

Methods: To assess proinflammatory versus anti-inflammatory T_H17 cell functions, we performed suppression assays and tested the effects of IL-1β dependent and independent T_H17 subsets on modulating proinflammatory cytokine secretion by monocytes. Patients with Schnitzler syndrome were analyzed for changes in T_H17 cell functions before and during therapy with IL-1β–blocking drugs.

Results: Both T_H17 cell subsets differ in their ability to suppress T-cell proliferation and their ability to modulate proinflammatory cytokine production by antigen-presenting cells because of their differential IL-10 expression properties. In patients with Schnitzler syndrome, systemic overproduction of IL-1β translates into a profound loss of anti-inflammatory T_H17 cell functionalities, which can be reversed by anti–IL-1β treatment.

Conclusion: IL-1β signaling determines the differential expression pattern of IL-10, which is necessary and sufficient to induce proinflammatory versus anti-inflammatory T_H17 cell functions. Our data introduce T_H17 cell subsets as novel players in autoinflammation and thus novel therapeutic targets in autoinflammatory syndromes including other IL-1β mediated

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diseases. This demonstrates for the first time alterations in the adaptive immune system in patients with autoinflammatory syndromes. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Autoinflammation, Schnitzler syndrome, T_H17 cells, IL-10, IL-1β*

Abbreviations used
FACS: Fluorescence-activated cell sorting
IL-1Ra: IL-1 receptor antagonist
Treg: Regulatory T

T_H17 cells have been recognized as critical players in the pathogenesis of autoimmune diseases and in the clearance of extracellular pathogens and fungi.[1,2] In mice terminal differentiation of T_H17 cells is dependent on IL-23, which abrogates the induction of IL-10 and promotes IFN-γ coexpression in IL-17–producing T helper cells. This confers pathogenicity to T_H17 cells, as evidenced most strikingly in the EAE mouse model of multiple sclerosis.[3] In human subjects 2 types of stable T_H17 cells have recently been identified *in vivo*.[4] They differ in their property to coexpress either IL-10 or IFN-γ and in their microbial antigen specificities. These distinct phenotypes are generated with differential requirements for IL-1β during the polarization phase. In humans IL-1β, but not IL-23, strongly suppresses IL-10 and promotes IFN-γ/T-bet induction.[4]

Despite this differential cytokine coexpression pattern, it has not been demonstrated so far whether these distinct human T_H17 cell phenotypes indeed translate into different functionalities. Although both subsets continue to express proinflammatory IL-17, the question of whether anti-inflammatory IL-10 might confer a dominant immunosuppressive functionality on T_H17 cells is warranted. This would necessitate a rethinking about the general concept of T_H17 cells being major mediators of inflammatory tissue destruction.

Autoinflammatory syndromes are characterized by overproduction of IL-1β caused by mutations in the inflammasome, a large intracellular multiprotein complex that leads to maturation of IL-1β and IL-18.[5] Due to the absence of inflammasome expression in cells of the adaptive immune system, autoinflammation has been considered to be caused solely by abrogations in innate immunity, although this could potentially also translate into downstream alterations of adaptive immune functions. Schnitzler syndrome belongs to this group of very rare autoinflammatory syndromes. It presents with a chronic urticarial rash, intermittent fever, arthralgia or arthritis, hepatomegaly and/or splenomegaly, lymphadenopathy, leukocytosis, and an increased erythrocyte sedimentation rate.[6,7] We recently demonstrated 2 variant cases with somatic mosaicism of *NLRP3* mutations in the myeloid lineage, leading to spontaneous IL-1β secretion by PBMCs.[8] Furthermore, increased IL-1β secretion by LPS-stimulated PBMCs was reported.[9] Hence, apart from the monoclonal IgM gammopathy, the immunologic significance of which is unknown, and IL-1β overproduction, no immune-related abnormalities have been detected in patients with Schnitzler syndrome so far.[6]

Because IL-1β is the critical switch factor that determines whether T_H17 cells can coexpress IL-10,[4] Schnitzler syndrome represents an "experiment of nature" in which alterations in adaptive immunity caused by systemic IL-1β overproduction can be studied *in vivo* in human subjects. In addition, immunologic perturbations, such as systemic *in vivo* inhibition of IL-1β by targeted therapies with IL-1 receptor antagonist (IL-1Ra; anakinra) and IL-1β–neutralizing antibodies (canakinumab and gevokizumab), can be performed *in vivo* in this disease setting.

This revealed the existence of 2 functionally distinct subsets of human T_H17 cells, which can be categorized into proinflammatory versus anti-inflammatory subsets because of the dominant role of IL-10 and despite the heterogeneous cytokine repertoire of both T_H17 cell subsets. We also demonstrate a loss of anti-inflammatory T_H17 cells in patients with Schnitzler syndrome, which could be restored to physiological levels upon therapeutic IL-1β inhibition. This could have implications for disease pathogenesis and future therapeutic strategies.

METHODS

Cell purification and sorting

$CD14^+$ monocytes and $CD4^+$ T cells were isolated from PBMCs after density gradient sedimentation with Ficoll-Paque Plus (GE Healthcare, Fairfield, Conn) by means of positive selection with CD14- or CD4-specific microbeads, respectively (Miltenyi Biotec, Bergisch Gladbach, Germany). T helper cell subsets were sorted to greater than 97% purity as follows: T_H1 subset, $CCR6^-CCR4^-CXCR3^+CD45RA^-CD25^-CD8^-$; T_H2 subset, $CCR6^-CCR4^+CXCR3^-CD45RA^-CD25^-CD8^-$; and T_H17 subset, $CCR6^+CCR4^+CXCR3^-CD45RA^-CD25^-CD8^-$. The following antibodies were used for fluorescence-activated cell sorting (FACS): phycoerythrin-conjugated anti-CCR6 (11A9; BD Biosciences, San Jose, Calif), phycoerythrin-indotricarbocyanine–conjugated anti-CCR4 (1G1; BD Biosciences), allophycocyanin-conjugated anti-CXCR3 (1C6/CXCR3; BD Biosciences), phycoerythrin-Cy5–conjugated anti-CD45RA (HI100, BD Biosciences), R phycoerythrin cyanin 5.1–conjugated anti-CD8 (B9.11; Coulter Immunotech, Krefeld, Germany), and fluorescein isothiocyanate–conjugated anti-CD25 (B1.49.9; Immunotech). Naive T cells were isolated as $CD45RA^+CD45RO^-CCR7^+CD25^-CD8^-$ to a purity of greater than 99% after staining with the aforementioned antibodies against CD45RA, CD25, and CD8, as well as fluorescein isothiocyanate–conjugated CD45RO (UCHL1; Immunotech) and anti-CCR7 (150503; R&D Systems, Minneapolis, Minn), followed by staining with biotinylated anti-IgG_{2a} (1080-08; SouthernBiotech, Birmingham, Ala) and streptavidin–Pacific blue (Molecular Probes, Invitrogen, Eugene, Ore). Permission for experiments with human primary cells was obtained from the ethics committee of the Charité-Universitätsmedizin Berlin (EA1/221/11), the ethics committee of the University of Wuerzburg (protocol no. 239/10), and the Radboud University Medical Center, Nijmegen, with approval by the local medical ethics committee, as well as from the ethics committee of the Technical University of Munich (195/15s). Samples from patients with Schnitzler syndrome from the NCT01390350[10] cohort were obtained before and at different time points after treatment with canakinumab during a proof-of-principle immunologic study at the Charité-Universitätsmedizin Berlin. Patients were eligible for enrollment in the study if they had active Schnitzler syndrome. All patients selected for this study responded to treatment with complete remission, as defined by physician's global assessment on overall autoinflammatory disease activity. For more information on clinical trial details, we refer to NCT01390350 and Krause et al.[10]

Samples from patients with Schnitzler syndrome after treatment with gevokizumab were obtained from the Nijmegen Center for Immunodeficiency and Autoinflammation. Clinical trial information is deposited in the EU Clinical Trials Register (EudraCT no. 2013-002562-39). PBMCs of patients with systemic juvenile idiopathic arthritis (Still disease) who were naive for any biologic treatment were obtained from the Laboratory of Pediatric Rheumatology and Special Immunology, University Children's Hospital, University of Wuerzburg and the German Registry for Autoinflammatory Diseases (AID-Net).