Immunology Letters 270 (2024) 106926

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Autoantibody mediated deficiency of IL-36-receptor antagonist in a subset of patients with psoriasis and psoriatic arthritis

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ARTICLE INFO

Keywords:
Autoantibodies targeting the IL-36 receptor antagonist (IL-36Ra) occur in subgroups of patients with PsA and Pso
IL-36Ra-autoantibodies deplete IL-36Ra serum/plasma level, result in immunecomplex formation, and promote unrestricted IL-36 signal

ABSTRACT

Objective: Psoriatic arthritis (PsA) is known as a seronegative form of spondylarthropathy. The interleukin-36 cytokine family may have a major role in disease pathogenesis and particularly the related cutaneous manifestations. In light of our recent observations on (transient) autoantibody phenotypes neutralizing endogenous anti-inflammatory receptor antagonists (progranulin, IL-1Ra) in different inflammatory conditions, we set out to investigate the potential role of such antibodies targeting IL-36 cytokine family members in PsA and psoriasis without arthritic manifestations (Pso).

Methods: In the present study we screened for hypothetic autoantibodies against the anti-inflammatory mediators IL-36 receptor antagonist (IL-36Ra) and anti-inflammatory IL-38 in PsA, Pso and inflammatory and healthy controls. Serum samples of patients with PsA ($n = 254$), Pso ($n = 100$), systemic lupus erythematosus (SLE, $n = 50$), rheumatoid arthritis (RA, $n = 100$), ulcerative colitis (UC, $n = 50$), Crohn's disease (CD, $n = 50$), and healthy controls ($n = 237$) were screened for autoantibodies against IL-36Ra and IL-38 as well as IL-36Ra levels by ELISA. Biochemical analysis for immune complexes and atypic protein isoforms as well as IL-36 signaling reporter assays were performed.

Results: Anti-IL-36Ra antibodies were detected in five out of 100 (5.0 %) patients with Pso, in 12 of 254 (4.72 %) patients with PsA and in one of 50 (2 %) patients with CD, but in none of the other investigated inflammatory or healthy controls. The IL-36Ra autoantibodies belonged to the IgG1 subclass and their titers ranged between 1:200 to 1:1600. They resulted in immune-complex formation, depletion of serum IL-36Ra levels and were functional in terms of facilitating unrestricted IL-36 signaling.

Conclusion: IL-36Ra autoantibodies were found in subgroups of patients with Pso and PsA and may drive respective pathology.

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https://doi.org/10.1016/j.imlet.2024.106926
Received 4 June 2024; Received in revised form 24 August 2024; Accepted 9 September 2024
Available online 10 September 2024
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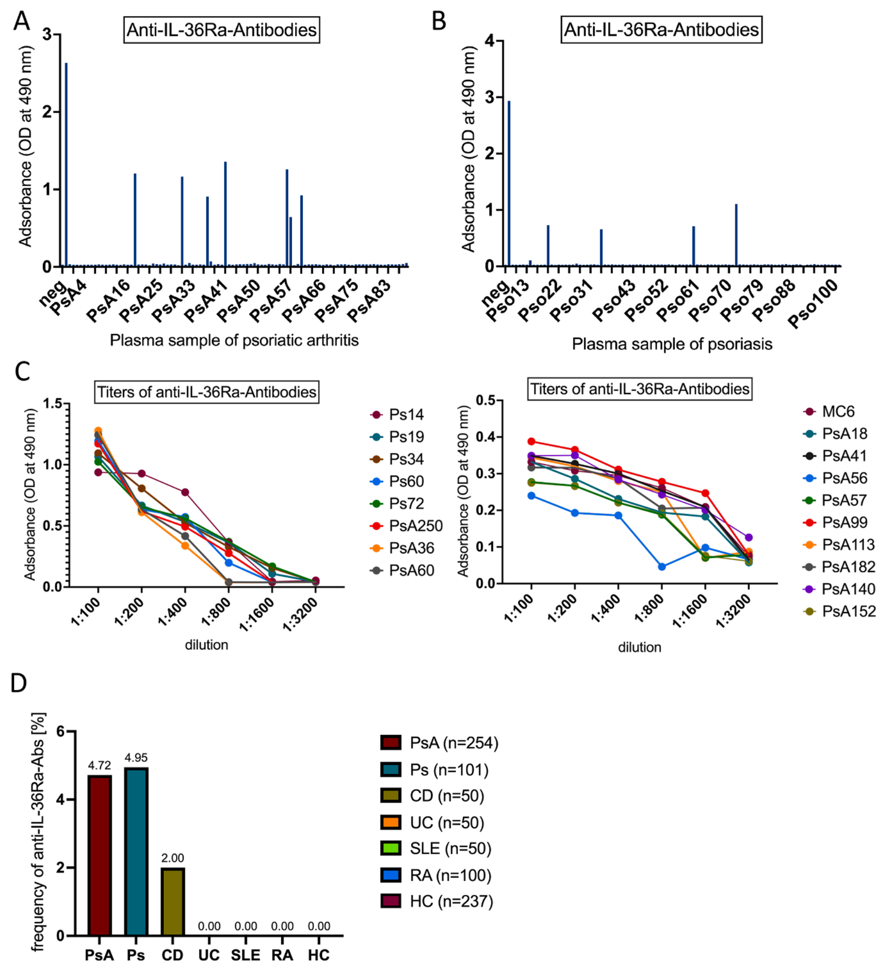

Fig. 1. Anti-IL-36Ra antibodies in psoriasis and psoriatic arthritis.
A, B) IL-36-Ra-autoantibodies were detected by ELISA in patients with Pso and PsA. **C)** Anti-IL-36Ra IgG titers were tested by ELISA. The titers ranged between 1:200 to 1:1600. **D)** Anti-IL-36Ra-autoantibodies Ig Frequencies of IL-36Ra-autoantibodies in Pso, PsA, autoimmune and healthy controls. $p = 0.0022$ comparing IL-36Ra-Abs in Ps with healthy controls; $p = 0.0005$ comparing IL-36Ra-Abs in PsA with healthy controls; $p = 0.0298$ comparing IL-36Ra-Abs in CD with healthy controls; two-tailed Fisher exact test.

1. Introduction

Psoriasis (Pso) and Psoriatic arthritis (PsA) are considered as sero-negative inflammatory diseases, diagnosed based on clinical presentation or histological results and can be linked with several genetic risk loci. Even though autoantibodies have been detected [1,2]. The most frequent autoantibodies detected in PsA to date are directed against the PsA peptide with an occurrence of 85 % [3]. Due to a shared homology of this PsA peptide epitope with antigens expressed in the skin and within the entheses, anti-PsA peptide antibodies are believed to interact with those autoantigens [3]. Other frequently detected autoantibodies in patients with PsA are the anti-carbamylated protein (anti-CarP) antibodies [2].

Dysregulated IL-36 pathway activation and signaling have an important role in cutaneous inflammatory manifestations. Neutrophil infiltration within the epidermis is - among others - stimulated by the interleukin (IL)−36 family. The IL-36 family of cytokines includes the pro-inflammatory cytokines IL-36α, IL-36β and IL-36γ as well as the anti-inflammatory mediators IL-36 receptor antagonist (IL-36Ra) and IL-38 [4]. IL-36Ra plays a critical role in the pathogenesis of generalized pustular psoriasis (GPP) which is a rare, life-threatening form of psoriasis. Mutations in the *IL36RN* gene like L27P can result in deficiency of IL-36 receptor antagonist (DITRA) representing one of the genetic causes of GPP [5].

Within the synovial tissues of patients with PsA, rheumatoid arthritis (RA) and osteoarthritis (OA) there is no difference between the expression of IL-36 receptor and IL-36Ra. Yet in comparison to OA, the expression of IL-36α has been found up-regulated in PsA and RA. Also, psoriatic skin lesions showed an increased gene expression of *IL36A, IL36G* and *IL36RN*. The agonistic IL-36 cytokines induce other proinflammatory cytokines, such as IL-6, IL-8 and TNF-α, which induce IL-36 as part of a positive feedback loop [6,7].

Next to genetic variations autoantibodies against anti-inflammatory mediators including receptor antagonists can disturb pro- *versus* anti-inflammatory immunological balance. In such lines, we previously described autoantibodies neutralizing Progranulin (PGRN) [8], a receptor antagonist at TNFR1, TNFR2 and DR3 [9], that occur with a prevalence of 19.23 % in patients with PsA [10].

More recently, we and others identified autoantibodies against the interleukin 1 receptor antagonist (IL-1Ra) in different inflammatory conditions [11–14]. Due to the central role of IL-36 family cytokines in the pathogenesis of Pso and PsA, in the present study we set out to screen for autoantibodies targeting the secreted anti-inflammatory receptor antagonists IL-1Ra, IL-36Ra and IL-38 in patients with Pso and PsA.

2. Material and methods

This study was approved by the local Ethical Review Board. Patient characteristics and data analysis are described in the supplement.

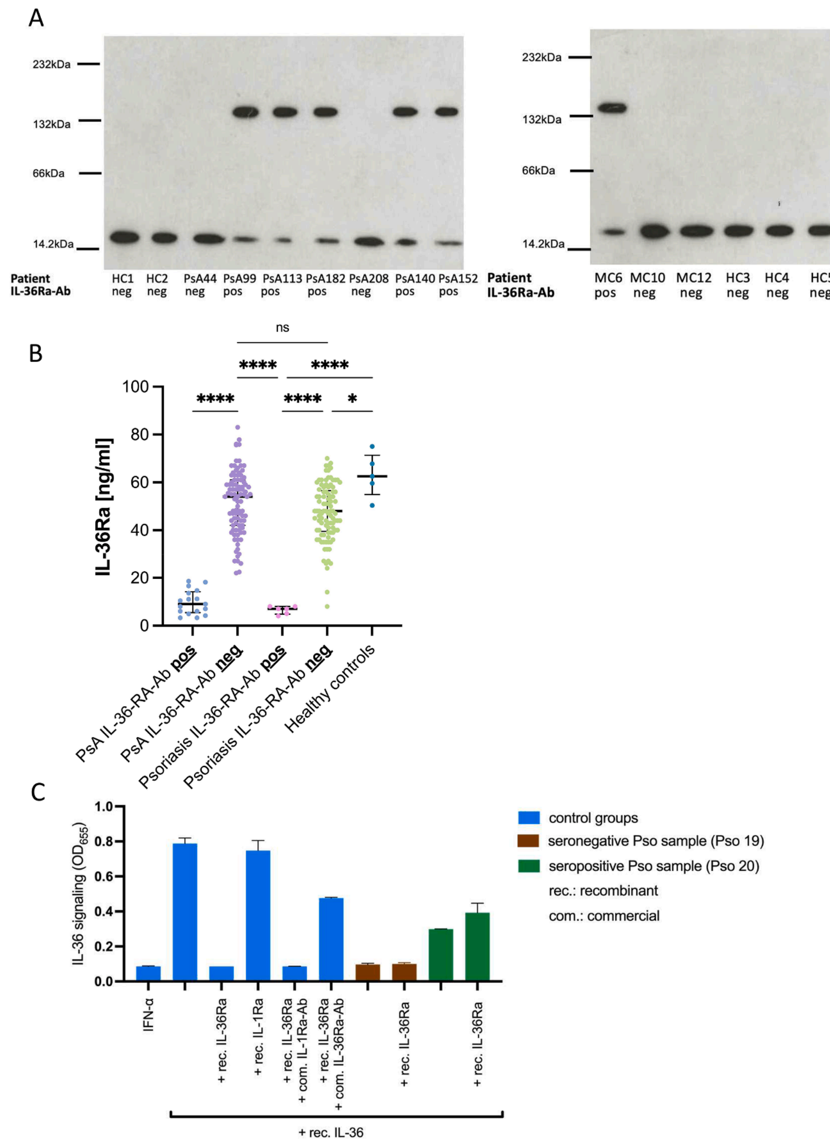

Fig. 2. Anti-IL-36Ra antibodies in psoriasis and psoriatic arthritis – immunocomplexes and neutralization.
A) Western blot of native gradient PAGE for IL-36RA:IgG immune complexes. Blood samples without IL-36Ra-autoantibodies had only one band for IL-36Ra with a molecular weight of around 14.2 kDa. Samples containing IL-36Ra-autoantibodies displayed two bands: one close to 150 kDa which indicated IgG-bound IL-36Ra, and a band of low signal intensity resembling free IL-36Ra at around 14.2 kDa. (**B**) Plasma levels of IL-36Ra determined by commercial ELISA according to autoantibody-status. Data are represented as individual values and were analyzed for normality distribution by Kolmogorov-Smirnov test. One way ANOVA and Tukey's multiple comparisons tests were used to compare the means of the IL-36Ra plasma levels in seropositive and seronegative patients. Lines indicate median and error bars the interquartile range. $^{**}P \leq 0.01$; $^{****}P \leq 0.0001$. Plasma samples of psoriasis and psoriatic arthritis with IL-36Ra-autoantibodies showed significantly lower plasma levels of free IL-36Ra compared to seronegative plasma samples. **C)** IL-36 signaling assay using HEK IL-36 reporter cells. The effects of different cytokines and antibodies on IL-36-signaling are displayed by the blue bars which serve as controls. The IL-36-Ra within the seronegative Pso blood sample mitigates IL-36 signaling. In comparison IL-36Ra-autoantibody containing Pso sample result in unrestricted IL-36 signaling due to neutralized receptor antagonist.

2.1. ELISA for autoantibodies against IL-36-Ra, IL-1Ra and IL-38

ELISA was performed with a system as described before. For this analysis C-terminally FLAG tagged IL-1Ra, IL-36Ra and IL-38 were used [11].

2.2. Western blot and isoelectric focusing of IL-36Ra

Isoelectric focusing (IEF) and Western blotting including native Western blotting with non-reducing sample pretreatment and gradient (4–20 %) native gels without SDS and native buffer are described in detail in the supplement.

2.3. ELISA for plasma level determination of IL-36Ra

IL-36Ra plasma levels were determined with a commercially available ELISA kit (AdipoGen #AG-46B-006KI01) according to the manufacturer's instructions.

2.4. IL-36 signaling reporter assay

For IL-36 assay HEK-Blue™ IL-36 reporter cells (Invivogen, hkb-hil36r) were used, which is described in detail in the supplement.

3. Results

3.1. Autoantibodies targeting anti-inflammatory mediators in Pso and PsA

The ELISA for anti-IL-36Ra-antibodies found autoantibodies in five of 100 (5.0 %) patients with Pso and in 12 of 254 (4.72 %) patients with PsA (Fig. 1A,B). In IL-36Ra-antibody-seropositive patients the titers ranged between 1:200 to 1:1600 (Fig. 1C). All detected anti-IL-36Ra-antibodies belonged to IgG1 subclass (Fig. 1D). Autoantibodies against IL-36Ra were found in one out of 50 patients with CD ($n = 50$; 1/50 (2 %)), but not in RA ($n = 100$), SLE ($n = 50$), and none of the enrolled 237 healthy controls (Fig. 1E and supplementary Figure 1N). The results of controls as well as of anti-IL-38-antibody ELISA are presented in the supplement.

3.2. Immune complexed IL-36Ra - but no atypic posttranslationally modified isoform

Western-blot revealed immune complexed IL-36Ra with IgG in IL-36Ra-autoantibody-seropositive samples (Fig. 2A). IL-36Ra-antibody-seropositive samples had significantly decreased IL-36Ra plasma levels (Fig. 2B). Employing IL-36 HEK reporter cells, this autoantibody-mediated depletion of circulating IL-36Ra resulted in impairment of IL-36Ra bioactivity and thus unrestricted IL-36 signaling (Fig. 2C and supplement). With important difference to our previous analysis on autoantibodies targeting IL-1Ra and PGRN in part in other disease context, in anti-IL-36Ra seropositive samples we found no evidence for atypical IL-36Ra isoforms at the level of electric charge as assessed by IEF (supplement).

4. Discussion

Here we report on the occurrence of autoantibodies targeting IL-36Ra in a small subpopulation (approximately 5 %) of patients with Pso or PsA. These IL-36Ra-autoantibodies belonged to the subclass IgG1. In seropositive samples their occurrence coincided with depletion of IL-36Ra plasma levels, immune-complexed IL-36Ra and impaired bioactivity. Despite the fact that these autoantibodies are only present in a small subset of patients, given the central role of IL-36Ra in Pso and PsA pathogenesis we speculate that an antibody-mediated IL-36Ra depletion may be pathogenetically relevant.

The inhibition of the truncated, biologically more active forms of the IL-36 agonists requires a molar excess of IL-36Ra [15]. Therefore, a slight decrease in IL-36Ra levels might significantly disturb the IL-36: IL-36Ra balance and compromise its biological antagonizing function. The central role of IL-36Ra for inflammatory skin disease is visible in a dramatic way in generalized pustular psoriasis (GPP) a rare, life-threatening form of psoriasis, which can be caused by mutations in *IL36RN* resulting in DITRA [5].

In contrast to our previous observations of atypically modified antigenic isoforms in patients with PGRN- or IL-Ra-autoantibodies [12, 16], or of B-cell receptors of specific mature B cell malignancies [17], no such immunogenic isoform of IL-36Ra could be found. However, our analysis may be limited by the fact that we could only access IL-36Ra from peripheral blood and not skin as the primarily affected tissue.

While our data may suggest a pathogenic role for IL-36Ra autoantibodies in a subgroup of patients with Pso or PsA resulting in a proposed form of "autoimmune DITRA", our findings need to be interpreted in the light of several limitations. Due to the retrospective nature of this study, we had only limited access to data on clinical characteristics including disease activity, treatment at time of sampling and response. Next, we did not have genetic information on *IL36RN* of these patients. Therefore, it is necessary to validate and investigate these findings in prospectively collected and well-characterized cohorts also including patients with GPP and controls of other diseases with prominent cutaneous manifestations.

CRediT authorship contribution statement

Marie-Christin Hoffmann: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Natalie Fadle:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Evi Regitz:** Writing – review & editing, Supervision, Methodology, Investigation. **Igor Age Kos:** Writing – review & editing. **Onur Cetin:** Writing – review & editing. **Vadim Lesan:** Writing – review & editing. **Klaus-Dieter Preuss:** Writing – review & editing, Supervision, Methodology. **Marina Zaks:** Writing – review & editing, Data curation. **Elisabeth Stöger:** Data curation. **Vincent Zimmer:** Writing – review & editing, Data curation. **Philipp Klemm:** Writing – review & editing, Data curation. **Gunter Assmann:** Writing – review & editing, Data curation. **Jochen Pfeifer:** Writing – review & editing. **Joerg Thomas Bittenbring:** Writing – review & editing, Data curation. **Moritz Bewarder:** Writing – review & editing. **Thomas Vogt:** Writing – review & editing, Data curation. **Claudia Pföhler:** Writing – review & editing, Data curation. **Bernhard Thurner:** Writing – review & editing. **Christoph Kessel:** Writing – review & editing, Supervision, Methodology. **Lorenz Thurner:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lorenz Thurner reports financial support was provided by Saarland University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imlet.2024.106926.

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