Negative association between autoantibodies against IL-17, IL-17/anti-IL-17 antibody immune complexes and destruction in rheumatoid arthritis

Autoantibodies against proinflammatory cytokines such as interleukin (IL)-1α are protective and a marker of good prognosis in rheumatoid arthritis (RA). They bind their antigen, that is, the cytokine, forming immune complexes (ICs). IL-17 is a new therapeutic target for a growing number of disorders, and levels of circulating bioactive IL-17 are associated with RA severity. Our objective was to define the contribution of anti-IL-17 autoantibodies and IL-17-anti-IL-17 ICs.

A competitive ELISA was developed to measure anti-IL-17 autoantibodies. A positive control with added anti-IL-17 antibodies showed an inverse dose–response curve reflecting the competition, with no variation with irrelevant antibodies (figure 1A). Plasma of 30 healthy donors were first tested to determine the threshold (absorbance=0.9±0.1 at 1/2 dilution) with no

Figure 1  Illustrations showing the detection of anti-interleukin (IL)-17 autoantibodies in the plasma of patients with rheumatoid arthritis (RA) versus healthy donors. Anti-IL-17 autoantibodies in plasma were detected with a competition ELISA (A). Plasma from patients with RA and healthy donors were first preincubated with horse serum overnight to prevent cross-reactivity with RF present in a large number in RA plasma. Plasma at 1/4, 1/8 and 1/16 dilutions were then incubated with 30 µL of IL-17A (50 ng/mL) (IL-17A, Dendritics, Lyon, France). After 1 h incubation, an exogenous mouse anti-human IL-17 detection antibody (406G9.02-HRP, Dendritics, Lyon, France) was added, to compete with the anti-IL-17 autoantibodies present in the plasma sample. This mixture was transferred to 96-well plates coated with a mouse anti-human IL-17 antibody (408H6.01, Dendritics, Lyon, France) for 2 h. Tetramethylbenzidine (TMB) substrate was added and absorbance was read at 620 nm. For the validation step, positive and negative controls were tested. Incidence of anti-IL-17 antibodies in 60 patients with RA classified as 30 destructive RA and 30 non-destructive RA was compared with 30 healthy donors (B).
variation between 1/4 and 1/8 dilutions, indicating an absence of anti-IL-17 autoantibodies. In contrast, they were detected in 36.6% of the 60 patients with RA (p<0.05 vs controls, figure 1B). To study the relationship with severity, 30 destructive RA (wrist Larsen score: 2 and over) versus 30 non-destructive RA (Larsen score: 0 or 1) were tested. The two groups had similar mean age (71.3±9.3 vs 66.1±10.8 years), disease duration (23.0±9.8 vs 18.6±9.6 years), DAS28 (4.0±1.4 vs 3.9±1.2) but a different level of bone destruction (Larsen score: 0.5±0.5 vs 3.3±1.0, p<0.0001). Anti-IL-17 autoantibodies were detected in 46.6% of the non-destructive RA versus 24.2% of the destructive RA (p<0.05), indicating a link with a better prognosis.

Having detected free anti-IL-17 antibodies, we next looked for bound antibodies in circulating ICs using a new ELISA (figure 2A). First, we had to produce a human anti-human IL-17 monoclonal antibody, obtained from circulating B cells from a patient with RA (14F7 Ab). IL-17 alone or with an irrelevant antibody gave no signal. Addition of IL-17 to form 14F7 Ab-IL-17 ICs increased the signal (OD=0.09±0.01 with 14F7 Ab alone vs 0.26±0.01 with 14F7 Ab+IL-17, p<0.03) (figure 2B). At high 1/64 to 1/1024 dilutions, IC detection showed a linear dose–response curve (figure 2C). Dilution curves with healthy donor samples showed a rapid decrease with low background detection, indicating the absence of IC. That dilution curve was not very different from that of the whole RA population (mean OD at 1/1024 dilution: 0.21±0.10 for healthy donors vs 0.30±0.19 for patients with RA, p=0.2). However, the dilution curve of the non-destructive RA samples was different from that of the controls (p<0.03). Higher titres of IC were detected in non-destructive RA versus destructive RA (OD±SD: 0.47±0.20 vs 0.23±0.10, respectively, p=0.004 at 1/128 dilution), indicating a negative correlation between IC titres and severity.

Samples were tested for circulating bioactive IL-17 detection, exactly as recently described. Dot plot analysis between bioactive IL-17 and IC shows that in samples with a level of IC over a 0.4 optical density, bioactive and thus free IL-17 was not detected (figure 2D). Conversely, samples with bioactive IL-17 contain low levels of IC. Similar observation was obtained with IL-17 and anti-IL-17 autoantibodies.

This is the first report on the regulation of circulating IL-17 function by autoantibodies. In non-destructive RA, autoantibodies were present in excess, binding IL-17 and forming ICs. This would lead to an absence or low levels of bioactive IL-17, as observed with the bioassay for bioactive IL-17. Conversely in destructive RA, high levels of free IL-17 can be bioactive and contribute to systemic inflammation and destruction. The correlation study confirmed that patients with non-destructive RA are defined by low IL-17, high anti-IL-17 and abundant ICs as compared with destructive RA (0.06±0.11 vs 0.67±0.72 for bioactive IL-17; 46.6% vs 24.2% positive for anti-IL-17 autoantibodies; 0.47±0.20 vs 0.23±0.10 for IC titres at 1/128 dilution) (figure 2D). This could explain the heterogeneous results of anti-IL-17 trials in RA.
Detection of anti-IL-17 autoantibodies and IC combined with bioactive IL-17 may represent biomarkers of interest to predict response to IL-17 inhibition.\(^8\)\(^9\)

Ndione Ndongo-Thiam,\(^1\) Alice Clement,\(^1\) Jean-Jacques Pin,\(^2\) Diane Razanajaona-Doll,\(^2\) Pierre Miossec\(^1\)

\(^1\)Immunogenomics and Inflammation Research Unit EA 4130 and the Department of Clinical Immunology and Rheumatology, University of Lyon, Lyon, France

\(^2\)Dendritics, Bioparc Laennec, Lyon, France

Correspondence to Professor P Miossec, Clinical Immunology Unit, Departments of Immunology and Rheumatology, Hôpital Edouard Herriot, Lyon Cedex 03 69437, France; miossec@univ-lyon1.fr

Contributors NN-T: experiments and writing; AC, J-JP and DR-D: development of assays and PM: concept and writing.

Funding NN-T is supported by the IHU prometteur OPERA. PM is a senior member of and supported by the Institut Universitaire de France.

Competing interests NN-T and PM hold a patent on the determination of bioactive IL-17 and have filed a patent for these results.

Patient consent Obtained.

Ethics approval Ethics committee of the hospitals of Lyon.

Provenance and peer review Not commissioned; externally peer reviewed.

Negative association between autoantibodies against IL-17, IL-17/anti-IL-17 antibody immune complexes and destruction in rheumatoid arthritis

Ndienne Ndongo-Thiam, Alice Clement, Jean-Jacques Pin, Diane Razanajaona-Doll and Pierre Miossec

Ann Rheum Dis 2016 75: 1420-1422 originally published online April 29, 2016
doi: 10.1136/annrheumdis-2016-209149