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

Autoantibodies against proinflammatory cytokines[1] such as interleukin (IL)-1α are protective and a marker of good prognosis in rheumatoid arthritis (RA).[2 3] 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,[4] and levels of circulating bioactive IL-17 are associated with RA severity.[5] 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

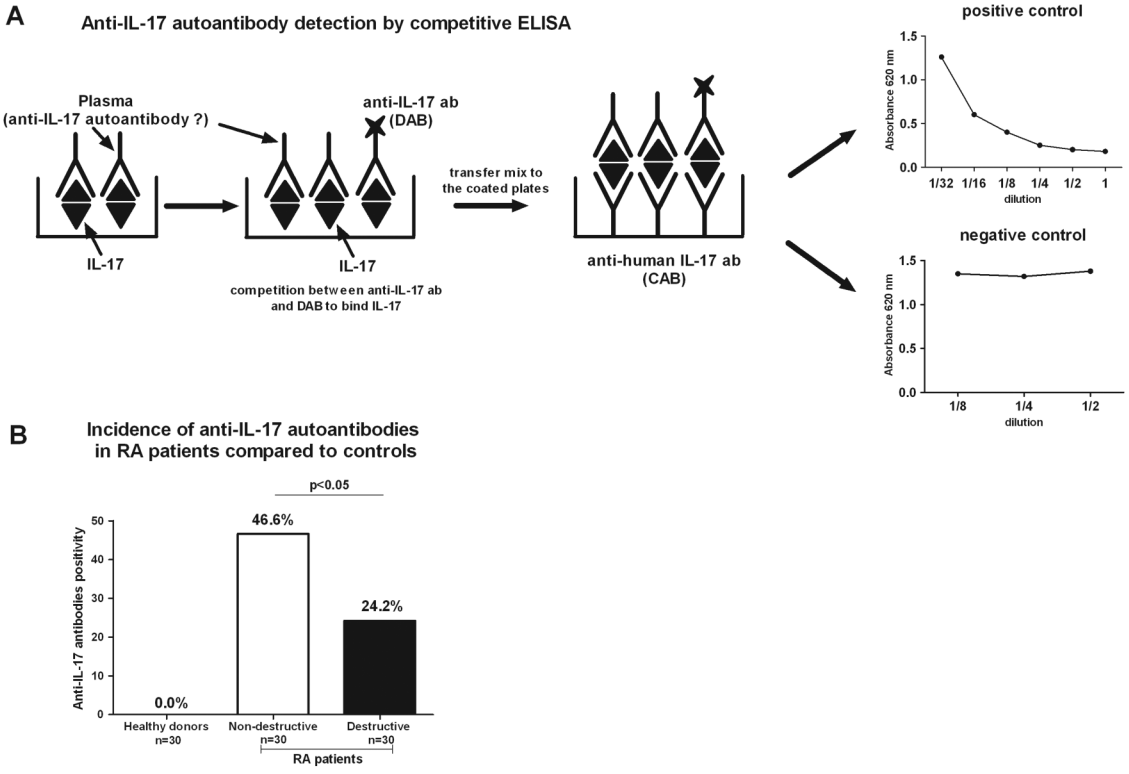

Figure 1 Illustrations showing the detection of anti-interleukin (IL)-17 autoantibodies in the plasma of patients with rheumatoid arthritis (RA) versus healthy donors. Anti-IL-17 autoantibodies in plasma were detected with a competition ELISA (A). Plasma from patients with RA and healthy donors were 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).

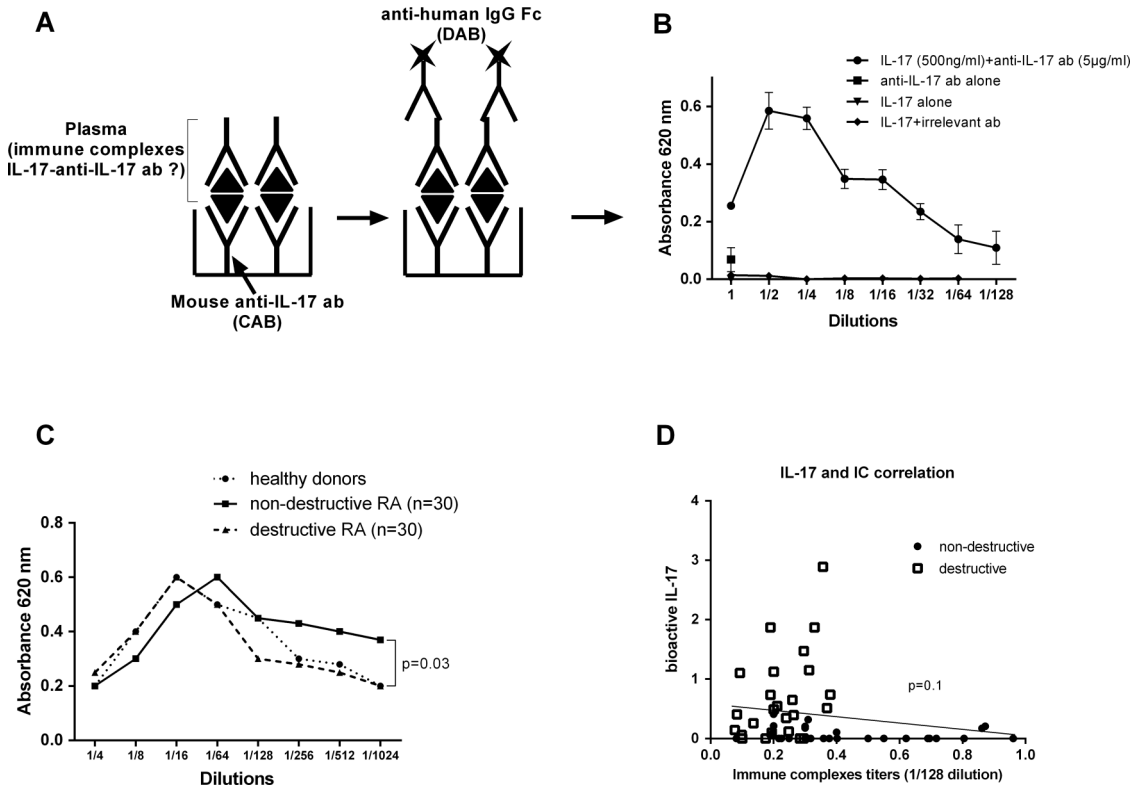

Figure 2 Detection of IL-17/anti-IL-17 antibody complexes in the plasma of non-destructive and destructive rheumatoid arthritis (RA) patients compared with healthy donors. An indirect ELISA was performed to detect IL-17/anti-IL-17 immune complexes (IC) in plasma, (A). Wells were coated overnight with 3 µg/mL of an anti-IL-17 capture antibody (408H6.01). After washing with PBS/Tween 0.05%, 100 µL of plasma dilutions were added overnight (first dilution at 1/2 in horse serum and 1/2 dilution in PBS/BSA/Tween). After three washes, a peroxidase-conjugated goat anti-human IgG Fc fragment specific antibody (109-035-098, Jackson Immuno research, Baltimore, USA) at 1/5000 dilution was added and incubated for 1 h 30. The positive IC control was made of a monoclonal human anti-human IL-17A antibody isolated from the blood of a RA patient (5 µg/mL) and IL-17 at 500 ng/mL. This human anti-human IL-17 antibody was obtained after immortalisation of human B lymphocytes from PBMC of RA patients with EBV and CD40 stimulation. (B) An eight point standard curve using twofold serial dilutions was tested with the positive IC control and plasma samples from 30 healthy donors and 60 RA patients: 30 with and 30 without bone destruction. (C). A correlation curve is represented in D between bioactive IL-17 and IC levels at 1/128 dilution.

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, $p<0.05$) (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.[5] 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, that is, free IL-17, as observed with the bioassay for bioactive IL-17.[5] 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.[6 7]

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]

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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.

To cite Ndongo-Thiam N, Clement A, Pin J-J, *et al*. *Ann Rheum Dis* 2016;**75**:1420–1422.

Received 8 January 2016
Revised 29 March 2016
Accepted 31 March 2016
Published Online First 29 April 2016

Ann Rheum Dis 2016;**75**:1420–1422. doi:10.1136/annrheumdis-2016-209149

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

Notes