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High *in vivo* expression of interleukin-17 receptor in synovial endothelial cells and chondrocytes from arthritis patients

M. C. Honorati[1,2], R. Meliconi[3], L. Pulsatelli[2], S. Canè[1], L. Frizziero[4] and A. Facchini[1,2]

[1]*Laboratorio Immunologia e Genetica, Istituto Codivilla Putti-IOR,* [2]*Dipartimento Medicina Interna e Gastroenterologia,* [3]*Dipartimento Medicina Interna, Cardioangiologia ed Epatologia, Università di Bologna and* [4]*Ospedale Maggiore, Bologna, Italy*

Abstract

Objective. To evaluate the presence of interleukin-17 (IL-17) and the expression of IL-17 receptor (IL-17R) in joint tissues from subjects with different arthritides.

Methods. Immunohistochemistry was used on frozen synovial and cartilage biopsies to identify cells expressing IL-17 and IL-17R.

Results. IL-17 staining was present only in synovial biopsies of rheumatoid arthritis (RA) (seven out of nine cases). IL-17R was expressed by all synovial biopsies evaluated except for three cases of post-traumatic arthritis (PTA). Vascular endothelial cells mainly expressed IL-17R. The percentage of IL-17R$^+$ vessels was the highest in RA synovium and the lowest in PTA. Chondrocytes from all types of arthritides were negative for IL-17 staining, but expressed IL-17R; the highest percentage of positive chondrocytes was found in seronegative spondylarthritis and the lowest in RA.

Conclusions. IL-17-positive cells are found exclusively in RA. On the other hand, synovial endothelial cells and chondrocytes expressing IL-17R are found in the majority of patients with different types of arthritis. This finding suggests a role for a second ligand for IL-17R, which could be either a different cytokine or a different isoform of IL-17.

KEY WORDS: Interleukin-17 receptor, Synovium, Endothelial cells, Chondrocytes, Arthritis.

A new human cytokine has been described recently, interleukin (IL)-17 [1], produced mainly by CD4$^+$ T lymphocytes [2]. It has been shown that IL-17 promotes the secretion of IL-6, IL-8 and granulocyte–macrophage colony-stimulating factor (GM-CSF) by fibroblasts [3] and IL-1β and tumour necrosis factor α by human macrophages [4]. In addition, this cytokine is active on endothelial cells [3] and up-regulates the production of nitric oxide by osteoarthritis (OA) chondrocytes [5, 6]. These results suggest that IL-17 has a role as a proinflammatory cytokine. As the presence of IL-17-positive cells has been described only in rheumatoid arthritis (RA) synovium [7, 8] and synovial fluid levels of IL-17 are much higher in RA than in OA [8], the role of this cytokine could be pivotal in RA. On the other hand, it is not known whether IL-17 is involved in the joint inflammation and damage that occurs in different arthritides, such as seronegative spondylarthritis (SA) and post-traumatic arthritis (PTA).

The structure of the human IL-17 receptor (IL-17R) has been characterized [9]. The presence of IL-17R has been demonstrated on the surface of various cells of human origin, such as NK cells from peripheral blood mononuclear cells, the Raji B cell line and human foreskin fibroblast cells, and it can be considered a ubiquitous receptor [9]. So far no data are available about which type of cells in the synovium express IL-17R. Endothelial cells from other anatomical sites are susceptible to IL-17 stimulation [3], and this cytokine could activate endothelial cells, thus supporting leucocyte extravasation and tissue infiltration.

Chondrocytes in culture are sensitive to IL-17 [5, 6, 10], but no data are available on the *in vivo* expression of its receptor on chondrocytes from donors with different types of arthritis. Therefore, the aim of this study was to evaluate the *in vivo* expression of IL-17R in the joint components of patients with different types of arthritis.

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Correspondence to: M. C. Honorati, Laboratorio di Immunologia e Genetica, Istituto di Ricerca Codivilla Putti-IOR, Via di Barbiano 1/10, 40136 Bologna, Italy.

Patients and methods

Patients

Arthroscopic synovial biopsies were obtained during diagnostic procedures from nine patients with RA (three males; mean age 51 yr, range 39–61); five patients with seronegative SA (two males; mean age 43 yr, range 26–60), including three with psoriatic arthritis (PsA); six patients with OA (one male, mean age 58 yr, range 53–64); and five patients with post-traumatic persistent knee pain (two males; mean age 32 yr, range 18–58). Arthroscopic cartilage biopsies were obtained from the same nine RA subjects, nine SA (five from the synovial biopsy group) (five males; mean age 53 yr, range 26–73) and 10 from OA cases (six from the synovial biopsy group) (three males; mean age 61 yr, range 26–60). The diagnosis was based on clinical, laboratory and radiological findings [11, 12]. Each patient gave informed consent and the study was approved by the ethics committees of the hospitals involved.

Biopsies

Local anaesthesia was administered. Then, using a Hamou–Storz microarthroscope (Karl Storz, Tuttlingen, Germany) as described previously [13], a synovial sample was obtained from the medial tibiofemoral gutter and a cartilage sample from a non-weight-bearing area of the medial condyle. The specimens were immediately covered with OCT medium, frozen in liquid nitrogen and maintained at -80°C until use.

Materials

Monoclonal murine antibodies (mAbs) anti-IL-17 (M68) and anti-IL-17R (M202) were kindly provided by Immunex Corporation (Seattle, WA, USA). Commercial materials were obtained from the following suppliers: anti-CD3 and anti-CD68 mAbs, rabbit antibodies to von Willebrand factor, pig alkaline phosphatase-labelled anti-rabbit immunoglobulin antibodies (anti-rabbit/AP), polyclonal mouse antibody isotype control and a new fuchsin substrate solution from Dako (Glostrup, Denmark); goat biotinylated polyclonal anti-mouse immunoglobulin antibody from KLP (Gaithersburg, MD, USA); OCT medium and levamisole from Sigma (St Louis, MO, USA); and alkaline phosphatase-labelled streptavidin (streptavidin/AP) from Boehringer Mannheim (Mannheim, Germany).

Immunohistochemistry

Frozen synovial or cartilage biopsies were cut with a cryostat into sections 4 μm thick, air-dried and stored at -80°C until use. Silanized slides were used for cartilage sections. Cryostat sections were fixed in acetone at 4°C for 10 min, dried and rehydrated. IL-17, IL-17R, CD3 and CD68 were revealed by an indirect method: sections were incubated overnight at 4°C with 10 μg/ml (synovial sections) or 20 μg/ml (cartilage sections) primary specific mouse monoclonal antibody, followed by incubation with 5 μg/ml secondary polyclonal biotinylated goat anti-mouse immunoglobulin antibody. The reacting antibodies were detected using streptavidin/AP at a dilution of 1:4000. To eliminate non-specific staining due to goat anti-mouse immunoglobulins, before each assay the slides were incubated for 10 min at 37°C with 5% goat normal serum and all other incubations were performed in the presence of 1.5% normal goat serum. Vascular endothelial cells were located in synovial sections by revealing von Willebrand factor by a first incubation of slides with rabbit-specific immunoglobulins at a dilution of 1:700, followed by a second incubation with anti-rabbit/AP at a dilution of 1:20. A new fuchsin substrate solution was used to develop the reactions, supplemented with 7.25 mM levamisole to block endogenous alkaline phosphatase. Each incubation with antibodies was for 30 min at room temperature, except the first described above.

A negative staining control was performed by omitting antibody from the first incubation. Non-specific reaction due to the isotype of the antibody was excluded by the use of mouse polyclonal immunoglobulins of the same isotype as that used for specific reactions.

IL-17R expression by vascular endothelial cells and chondrocytes

The percentage of IL-17R-positive vessels was calculated in whole sections after counting, for each synovial biopsy, the total number of vessels (von Willebrand factor-positive vessel endothelium or cell clusters) and the total number of vessel endothelium or cell clusters positive for IL-17R expression in serial sections. The percentage of IL-17R-positive chondrocytes was calculated by counting the total number of cells and the number of stained cells.

Statistical analysis

Differences between percentages were analysed by Fisher's exact test. Differences between means were analysed by Student's *t*-test. Pearson's correlation coefficients were calculated.

Results

IL-17 expression

IL-17 staining was present only in RA synovial biopsies (seven out of nine cases, 78%). No staining was observed in SA, OA and PTA synovial samples. In positive RA synovium, the few IL-17-positive cells were scattered in a dense lymphoid infiltrate; rarely they formed aggregates of three to five cells (4% $IL\text{-}17^+$ cells/$CD3^+$ cells, range 0–8.8%) (Fig. 1). No chondrocyte staining was observed in any of the patient groups examined.

IL-17R expression

Synovial membrane. Unlike the IL-17 results, receptor expression was found in synovial biopsies from all diseases evaluated. All inflammatory arthropathies (RA, SA) and OA cases were IL-17R-positive; on the contrary, only two out of five PTA cases expressed

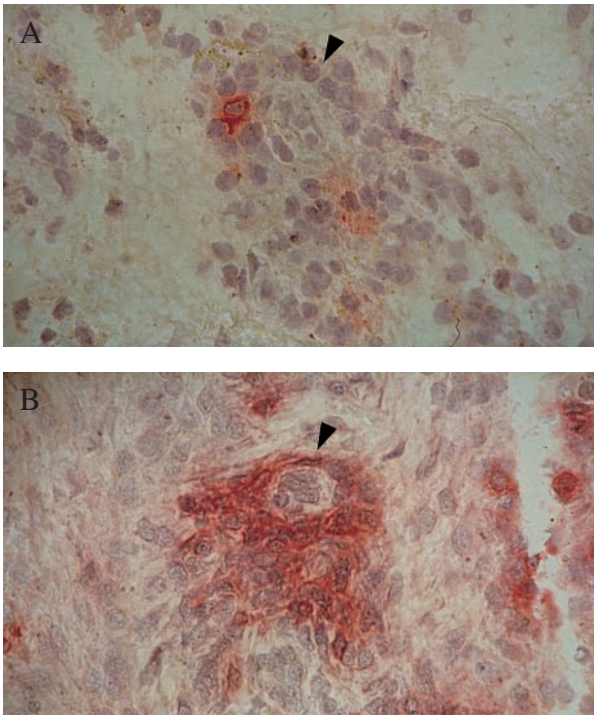

FIG. 1. Immunohistochemical staining for IL-17 in RA synovial section. (A) Staining with anti-IL-17. (B) Staining with anti-CD3. Arrowheads indicate blood vessels. Original magnification × 500.

IL-17R ($P < 0.05$ compared with RA). Expression of IL-17R was localized in the sublining area; the receptor was mainly expressed by vascular endothelial cells, and its presence did not correlate with the severity of lymphoid infiltrate (Fig. 2). The percentage of IL-17R-positive vessels was high in RA samples; it was significantly lower in OA and PTA cases, and SA samples showed an intermediate percentage (Fig. 3). No correlation was found between the number of IL-17-positive cells and the percentage of IL-17-positive vessels.

Cartilage. Chondrocytes showed *in vivo* expression of IL-17R in all types of arthritides (Fig. 4). The highest percentage of positive chondrocytes was in SA and OA, and the lowest in RA (Fig. 5).

Discussion

In the present report we confirm that IL-17 is produced only in RA synovial membrane, all the other arthropathies being negative. Furthermore, we report the *in vivo* expression of IL-17R. Our results are surprising because (i) we found a much broader expression of IL-17R than of IL-17, the receptor being expressed in almost all synovial specimens studied; (ii) IL-17R was expressed almost exclusively by endothelial cells in synovium; and (iii) IL-17R was expressed by the vast majority of cartilage biopsies examined, the lowest percentage of positive chondrocytes being found in RA samples. Other authors have described the presence of positive IL-17 synovial cells in RA but not in OA [7, 8]. Our results confirm these previous reports and strengthen the argument for the exclusiveness of IL-17 expression in RA. We also indicate that seronegative SA and PTA do not show synovial staining for IL-17.

In rheumatoid synovial tissue, IL-17 staining is exclusively located in CD3-rich areas. In addition, we observed that the number of IL-17-producing T cells is extremely low, even in samples with dense lymphocyte infiltrate. Due to its various proinflammatory actions, IL-17 seems to contribute to the chronicity and progression of RA. The problem is to establish why IL-17-producing cells are present only in RA synovium. The discrepancy in RA synovium between the presence of activated DR-positive memory T cells [14] and the paucity of IL-2 and interferon γ-producing cells [15–18] led to the 'frustrated T-cell' hypothesis [19, 20]. Recently IL-15 has been identified in RA synovium [21], and it is known that this cytokine triggers the production of IL-17 by peripheral blood mononuclear cells [22]. Therefore, we suggest that IL-17 could represent the major T_H1 $CD4^+$ product in RA synovium. This different usage of T-cell stimulatory factors in RA could be also dependent on the antigenic stimulus inducing the rheumatoid immunopathology [23], associated with HLA class II determinants [24–26]. If our data on the histological expression of IL-17 are confirmed in a larger series of rheumatoid *vs* non-rheumatoid synovial biopsies, the positivity of IL-17 could be a histological diagnostic marker for RA.

Previous indirect *in vitro* evidence of IL-17R expression was obtained in a variety of cell types: fibroblasts and fibroblast-like synoviocytes [2, 3], epithelial and endothelial cells [3], keratinocytes [27, 28], macrophages [4], osteoclasts [8] and stromal cells [3]. In the joints, it has been demonstrated that synoviocytes and chondrocytes respond to IL-17 stimulation with induced production of proinflammatory cytokines and iNOS (inducible nitric oxide synthase), cyclooxygenase-2 and stromelysin [3–6, 10]. In our study, the *in vivo* expression of IL-17R was confined to synovial endothelial cells and chondrocytes. No staining of synoviocytes was observed either in the lining layer or in the sublining area. The lack of IL-17R staining in synovial fibroblast was surprising, and in disagreement with the above-mentioned functional studies. The combined lack of receptor expression and active functional response suggests the presence of a second receptor on fibroblasts and synoviocytes which is not recognized by the monoclonal antibody used in this study, being at the present time IL-17R and its ligand members of a new family of cytokines [29]. Many cytokines or growth factors recognize more than one receptor [30]. On the other hand, chondrocyte expression of IL-17R is in keeping with the results of previous studies [6, 10].

Even more interesting is the finding of IL-17R expression on endothelial cells and chondrocytes from patients negative for IL-17 staining in the synovium. Why should IL-17R be present if no IL-17 is produced locally? The presence of IL-17R on vessel endothelial cells suggests

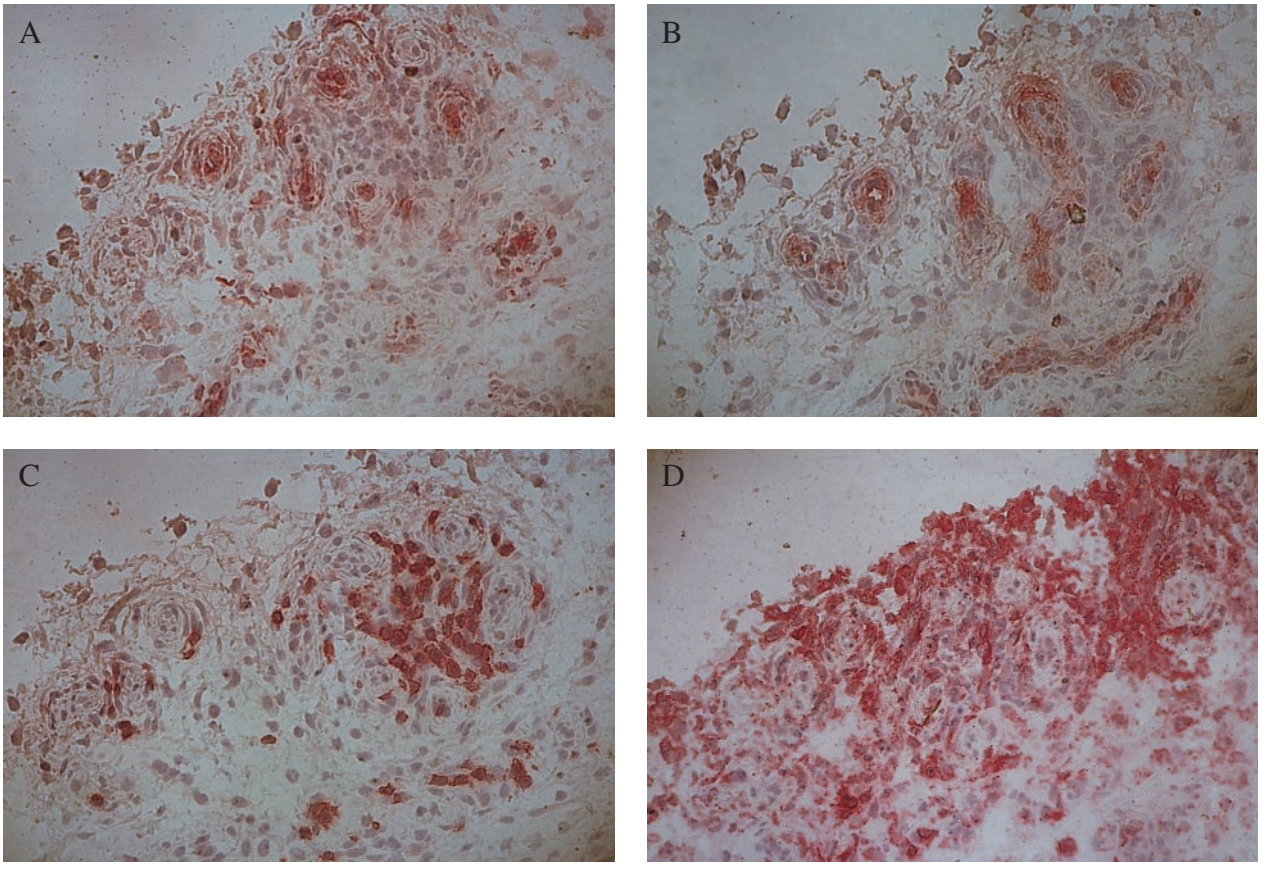

FIG. 2. IL-17R expression by vessel endothelial cells. Serial sections of synovial biopsy from a case of SA are represented. (A) Staining with anti-IL-17R. (B) staining with anti-von Willebrand factor. (C) Staining with anti-CD3. (D) staining with anti-CD68. Original magnification × 250.

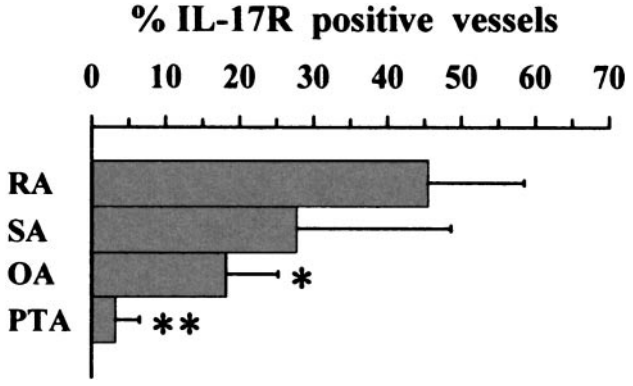

FIG. 3. Percentage of vessels positive for IL-17R. Results are expressed as mean of eight subjects with RA, four subjects with SA, four subjects with OA and four subjects with PTA. Horizontal bars represent S.E.M. $*P < 0.05$ *vs* RA; $**P < 0.01$ *vs* RA.

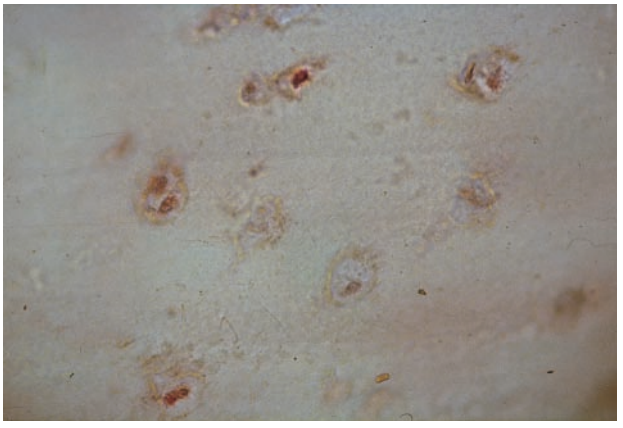

FIG. 4. IL-17R expression by chondrocytes. Section of cartilage from a subject with OA. Original magnification × 500.

that IL-17R could also be recognized by different, so far unknown, ligands. Receptor sharing is a well-established phenomenon in cytokine and, in particular, chemokine biology [31]. These ligands should be particularly active in OA and SA, which do not show IL-17 production. The very recent identification of two new members of the IL-17 family (IL-17B, IL-17C) could support our hypothesis [29]. The lower percentage of IL-17R-positive chondrocytes found in RA cartilage samples may suggest down-regulation of receptor expression in chondrocytes in the presence of relatively high levels of IL-17. On the other hand, these high IL-17 levels do

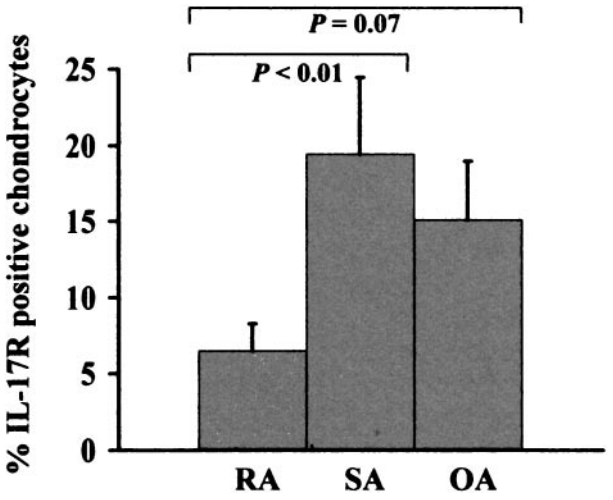

FIG. 5. Percentage of chondrocytes positive for IL-17R. Results are expressed as mean of nine subjects with RA, nine subjects with SA and 10 subjects with OA. Vertical bars represent S.E.M.

not down-regulate this expression on synovial endothelial cells. Therefore, different mechanisms of regulation of receptor expression could be operating in endothelial cells and chondrocytes, as for other cytokines [32, 33].

In conclusion, our study shows expression of IL-17R by endothelial cells and chondrocytes. In RA the high level of vascular expression is matched by low expression on chondrocytes; the opposite occurs in OA and SA. Studies of the regulation of receptor expression are of the utmost importance in elucidating the role of IL-17 in endothelial cell and chondrocyte biology.

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