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Cutting Edge: Mast Cells Express IL-17A in Rheumatoid Arthritis Synovium

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The proinflammatory cytokine IL-17A is considered a crucial player in rheumatoid arthritis (RA) pathogenesis. In experimental models of autoimmune arthritis, it has been suggested that the cellular source of IL-17A is CD4+ T cells (Th17 cells). However, little is known about the source of IL-17 in human inflamed RA tissue. We explored the cellular sources of IL-17A in human RA synovium. Surprisingly, only a small proportion of IL-17-expressing cells were T cells, and these were CCR6 negative. Unexpectedly, the majority of IL-17A expression was localized within mast cells. Furthermore, we demonstrated in vitro that mast cells produced RORC-dependent IL-17A upon stimulation with TNF-α, IgG complexes, C5a, and LPS. These data are consistent with a crucial role for IL-17A in RA pathogenesis but suggest that in addition to T cells innate immune pathways particularly mediated via mast cells may be an important component of the effector IL-17A response. The Journal of Immunology, 2010, 184: 3336–3340.

Understanding of the pathogenesis of rheumatoid arthritis (RA) has advanced substantially in recent years, driven in part by direct investigation of synovial tissue. Recently, the cytokine IL-17A has been linked to disease mechanisms. In rodent models, deficiency or blockade of IL-17A leads to suppression of arthritis (1). In humans, expression of IL-17A in RA synovium and synovial fluid was first described in 1999 (2). Initial phenotyping studies indicated that synovial T cells with plasmacytoid phenotypic characteristics expressed IL-17A. Most subsequent reports have focused on expression in peripheral blood, and few reports have studied Th17 cell markers in situ (3, 4). Th17 cells are a novel lineage of T helper/effector CD4 cells, selectively expressing the transcription factor RORγt (RORC gene product), that produce IL-17A, IL-17F, IL-22, CCL20, and other moieties, and are thought to account for initiation and maintenance of several inflammatory disorders (reviewed in Refs. 5, 6). Although there remains some controversy over the precise signals required for the development of murine and human Th17 cells, it is generally agreed that human CD4+ Th17 cells require IL-1, IL-6, and IL-23 for their proliferation and maintenance, cytokines that are detected in RA synovium. In human PBMCs, CCR6 and CCR4 are coexpressed on Th17 cells (7). CCR6 is thought to be involved in the recruitment of Th17 cells to sites of inflammation via the ligand CCL20.

Even though in vitro phenotypic characteristics and commitment of Th17 cells appear robust, in vivo data increasingly support plasticity of this lineage. Transfer of Th17 cells into an experimental autoimmune encephalomyelitis model resulted in a shift from Th17 to Th1 phenotype (8). Moreover, T regulatory cells may express IL-17A under certain conditions but still retain suppressive function reflecting a more plastic cell commitment (9). Other cell types including γδ T cells, NKT cells, NK cells, neutrophils, and eosinophils have been reported to produce IL-17A (5, 6), as have innate CD4+ lymphoid tissue inducer-like cells (10). Also recently, mast cells have been described to express IL-17A and IL-17F (11, 12). Thus, in tissue, the net IL-17A expression may arise from a broad array of adaptive and innate cells. For this reason and because IL-17A is now being targeted in clinical trials, we sought to re-evaluate the dominant cellular source of IL-17A in established RA synovium. We show that Th17 cells are relatively rare in RA synovium. However, whereas a small percentage of macrophages produce IL-17A, mast cells are proportionally the cells that express the highest level of IL-17A in the RA synovium. Moreover, in vitro studies reveal that mast cells produce IL-17A in response to various inflammatory stimuli, including TNF-α, IgG complexes, C5a, and LPS, all of which are upregulated or contribute to the pathology of the inflammatory synovium, or both. Thus, our results show mast cells as a key source of IL-17A in RA.
adding important evidence that mast cells in synovial tissue represent a promising target for RA treatment.

**Materials and Methods**

**Patient samples**

Synovial tissue from patients with RA (n = 10) and osteoarthritis (OA) (n = 4) were obtained from the Centre for Rheumatic Diseases (Glasgow, U.K.) derived by arthroplasty. American College of Rheumatology classification for RA was fulfilled. Written informed consent was given before inclusion in the study, under approval from the Glasgow East Ethics Committee.

Human umbilical cord blood samples were collected from normal full-term deliveries of informed individuals with formal consents, meeting the University Institutional Review Board guidelines (National University of Singapore, Singapore) for research using human samples.

**Fluorescent microscopy and immunohistochemistry**

Five-micrometer sections of RA synovium were deparaffinized and rehydrated. The 0.5% hydrogen peroxidase/methanol incubation and heat retrieval in 0.5 M citrate buffer (pH 6) was followed by incubation in 2.5% species/2.5% human serum with Avidin D (Vector Laboratories, Petersborough, U.K.). Staining for 1 h with Abs (mouse anti-CD3 [1.25 μg/ml; Vector Laboratories], mouse anti-CD4 [7.56 μg/ml; Dako UK, Cambridgehire, U.K.], rabbit anti-CCR6 [0.75 μg/ml; Sigma-Aldrich, Dorset, U.K.], mouse anti-mast cell tryptase [MCT] [0.43 μg/ml; Dako UK], or mouse anti-CD68 [1 μg/ml; Dako UK]) was followed by 30 min incubation with biotinylated Abs (1:200; Vector Laboratories) with subsequent staining with streptavidin QDot605 (1:250; Invitrogen, Paisley, U.K.) for 45 min. Goat anti–IL-17 (5 μg/ml; R&D Systems, Abingdon, U.K.) was added overnight at 4˚C, then incubated with a biotinylated Ab for 30 min, and stained with Avidin FITC (1:500; Vector Laboratories) for 45 min. Slides were mounted with Vectashield containing DAPI (Vector Laboratories) and analyzed on a fluorescent imaging microscope (BX50; Olympus, Essex, U.K.). Images were captured using Apple Open laboratory software.

For immunohistochemistry, prepared RA or OA sections were incubated with goat anti–IL-17 overnight, incubated with ImmPRESS reagent anti-goat Ig for 30 min (Vector Laboratories), and signal-developed using the peroxidase substrate nickel diaminobenzidine tetrahydrochloride (Vector Laboratories).

**Quantification of IL-17–expressing cells**

Images were captured digitally, and the total number of IL-17A+ cells was calculated that were found within duplicate tissue areas (two representative × 10 fields) of 0.52 mm2. Double staining allowed us to calculate the proportion of IL-17A–expressing cells per cell surface marker. Four tissues were double-stained for all markers, with confirmatory studies performed on a further six tissues to confirm the coexpression with MCT and CD68.

**Mast cell stimulation**

Human mast cells were derived from CD34+ hematopoietic progenitor cells isolated from umbilical cord blood and cultured as described previously (13, 14). Mast cells were stimulated with TNF-α (10 ng/ml), IgG complexes (100 ng/ml), C5a (5 ng/ml), or LPS (10 ng/ml) for 24 h, and supernatant was tested for IL-1β, IL-5, IL-6, and IL-17A by ELISA (ELISA kits from eBioscience, Hatfield, U.K.). IgG complexes were formed by heating 1 μg IgG to 60˚C for 15 min to generate aggregated IgG complexes. Average and SD are shown from three independent experiments.

**Real-time PCR**

Total RNA was isolated using an RNeasy kit (Qiagen, West Sussex, U.K.), and cDNA was synthesized using a High-Capacity cDNA Archive kit (Applied Biosystems, Warrington, U.K.). The primers for RORC and STAT3 were purchased from Applied Biosystems, and gene expression was measured by quantitative real-time PCR using TaqMan Gene Expression Assays (Applied Biosystems). Results are expressed for each gene as the relative gene expression normalized to the 18S mRNA expression. STAT3 gene expression was monitored as a control (Supplemental Fig. 2).

**Small interfering RNA transfection**

Ready-made RORC small interfering RNA (siRNA) and negative control siRNA were purchased from Qiagen. The siRNAs were transfected, using the HiPerFect Transfection Reagent from Qiagen, and 48 h later experiments were carried out. Expression levels were monitored by real-time PCR (Supplemental Fig. 2).

**Results and Discussion**

We first established the tissue location of IL-17A–expressing cells using light microscopy. Cytoplasmic IL-17A staining was observed in cells of an ovoid/plasmacytoid phenotype, as described previously (2), and in cells of a more irregular

**FIGURE 1.** IL-17A expression in RA synovium. Cells staining for IL-17A by immunohistochemistry show different cell morphology. Squares and arrows demonstrate different examples. Two fields (×40) of a representative RA synovium tissue are shown.

**FIGURE 2.** Th17 cells are hardly detectable in established RA synovium. Synovial tissue samples from RA patients (n = 10) were stained for IL-17A (green) and indicated markers. Sections were counterstained with DAPI. Th17 cell markers CCR6 and CD4 or CD3 (red) and macrophage marker CD68 (red) are shown at ×40 magnification. MCT (red) and IL-17A double staining are shown at ×10 (upper right panel) and ×40 (lower right panel) magnifications.
phenotype, both at the periphery of lymphocytic aggregates, in
the sublining layer areas, and rarely in the lining layer (Fig. 1).
Interestingly, IL-17A–positive cells were also occasionally de-
tected in OA tissue, but with low frequency (Supplemental Fig.
1). To formally identify these IL-17A+ cells in RA synovium,
we performed colocalization studies and calculated the pro-
portion of IL-17A+ cells contained in each cellular subset. We
first investigated a variety of T cell markers, namely, CD3,
CD4, and CCR6, colocalized with IL-17A. Although occa-
sional CD4+IL-17A+ cells were identified, the majority of IL-
17A+ cells were CD4-negative (<1%), consistent with the
notion that CD4+ Th17 cells represent a minority of the IL-
17A–producing population in synovitis (Fig. 2, upper left panel,
Table I). This also renders it unlikely that lymphoid tissue
inducer-like cells are major contributors, because these cells
express CD4 (10). Moreover, counterstaining with CCR6,
a previously defined marker for Th17, cells did not colocalize
with IL-17A (Fig. 2, upper middle panel). CD3+CD8+ T cells
have been shown to produce IL-17 (15). Of interest, only 1–
8% of IL-17A–expressing cells were found to express CD3
(Fig. 2, lower middle panel, Table I)—by inference from the
foregoing, these most likely comprise CD8+ cells or γδ T cells.
We next addressed the phenotypically distinct subset of IL-
17A+ cells of irregular shape. The latter initially suggested
macrophages as a potential lineage—IL-17A production by
macrophages has been suggested in allergic lung inflammation
(16). Consistent with this, up to 35% of IL-17A+ cells were
CD68+. However, because a substantial number of IL-17A
cells remained unattributed in these studies, we sought ad-
ditional cellular sources (Fig. 2, lower left panel, Table I).
Tissue-like mast cells have been reported to produce IL-17A
 upon TLR2 stimulation (17). To determine whether mast
cells might be an additional source for IL-17A, we colocalized
MCT and IL-17A (Fig. 2, upper and lower right panels, Fig.
3). The majority of IL-17A+ cells double-stained strongly with
MCT (46–100% of IL-17A–expressing cells per tissue area
across patients), clearly demonstrating that mast cells are key
producers of IL-17A in RA synovium (Table I).
The factors that might drive synovial mast cell IL-17A
expression have not previously been explored. RA synovial
membrane contains a number of candidate pathways that
could promote IL-17A expression, including complement

Table I. Percentage of double-positive cells compared with IL-17+ cells

<table>
<thead>
<tr>
<th>Cells per mm²</th>
<th>Percentage of IL-17+ Cells (Mean)</th>
<th>Percentage of IL-17+ Cells (Minimum/Maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3 analysis (n = 4)</td>
<td>CD3−IL-17+ 64</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>CD3−IL-17+ 2</td>
<td>3.4</td>
</tr>
<tr>
<td>CD68 analysis (n = 10)</td>
<td>CD68+IL-17+ 57</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>CD68+IL-17+ 6</td>
<td>9.8</td>
</tr>
<tr>
<td>MCT analysis (n = 10)</td>
<td>MCT−IL-17+ 8</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>MCT−IL-17+ 83</td>
<td>91</td>
</tr>
</tbody>
</table>

Absolute cell numbers (third column) and ratios to the total amount of IL-17+ cells (fourth column) for CD3−IL-17+ (n = 4), CD68+IL-17+ (n = 10), and MCT−IL-17+ (n = 10) cells are shown. Cell numbers were assessed in two independent fields with each ~0.5 mm².

![FIGURE 3. Mast cells express IL-17A in RA synovium. MCT (red) and IL-17A (green) are shown single-stained and merged with DAPI counterstain (left panel, ×40 magnification). This is further digitally magnified to demonstrate cellular structure (right panel).](https://www.jimmunol.org/)

![FIGURE 4. RORC-dependent IL-17A production by CD34+–derived mast cells. Cytokine release was determined from mast cells, untransfected (Δ) or siRNA-RORC–transfected (B), after 24 h stimulation with TNF-α, IgG complexes, C5a, and LPS, compared with a basal secretion (unstimulated cells). Cell culture supernatants were analyzed for bioactive IL-1b, IL-5, IL-6, and IL-17A by ELISA. RORC gene expression was determined by quantitative real-time PCR and silenced by siRNA targeting RORC (C). Results shown are the mean plus the SD of triplicate measurements of three separate experiments.](https://www.jimmunol.org/)
The crucial observation in our study was the colocalization of IL-17A with MCT, a highly specific marker for mast cells in rheumatoid arthritis. Their presence has long been retained as a major source of IL-17 in established RA synovium.

Our data refer only to established synovitis. Raza et al. (23) reported that in early compared with established RA synovial fluid IL-17A levels were significantly higher. The role that IL-17 cells play, as opposed to innate pathways, as a source for IL-17A in early arthritis therefore needs to be determined. With the emerging plasticity of Th17 cells, it is possible that their cytokine profile, and indeed chemokine receptor expression (particularly CCR6), might change upon entry to the tissue compartment or over the course of the disease. IL-17 blockade, in clinical trials, is suggested to have beneficial effects in psoriasis and in RA as a potential novel therapeutic, although formal appropriately designed and powered trials are required (24, 25). Interestingly, responses in such studies are fairly rapid, suggesting that the clinical effect may be mediated via blockade of the cytokine in circulation. Interestingly, our study strongly suggests that targeting mast cell responses may also lead to beneficial effects in RA, because activated mast cell contribute to the IL-17–rich environment and therefore may promote an amplification of innate responses in the joint (e.g., by activating neutrophils and by synergizing with TNF and IL-1 to drive increased cytokine/chemokine and eicosanoid synthesis). In conclusion, our study demonstrates that proportionally mast cells and not Th17 cells appear to be a major source of IL-17 in established RA synovium and that such production can be driven by a variety of pathways abundantly expressed therein.

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Disclosures

The authors have no financial conflicts of interest.

References


