INCREASED EXPRESSION OF INTERFERON (IFN)-GAMMA TOGETHER WITH IFN-GAMMA RECEPTOR IN THE RHEUMATOID SYNOVIAL MEMBRANE COMPARED WITH SYNOVIAL OF PATIENTS WITH OSTEOARTHRITIS

R. J. E. M. DOLHAIN,* N. T. TER HAAR,† S. HOEFAKKER,‡ P. P. TAK,§ M. DE LEY,¶ E. CLAASSEN,† F. C. BREEDVELD* and A. M. M. MILTENBURG*  

*Department of Rheumatology, University Hospital Leiden, †TNO Prevention and Health, Leiden, ‡Department of General Internal Medicine, University Hospital Leiden, Leiden, The Netherlands and §Laboratory for Biochemistry, Leuven University, Leuven, Belgium

SUMMARY

Data concerning the presence of T-cell-derived cytokines in the rheumatic joint are conflicting, challenging the hypothesis that rheumatoid arthritis (RA) is a T-cell-mediated disease. In this study synovial tissue specimens of 11 patients with RA and eight patients with osteoarthritis (OA) were stained for interferon-gamma (IFN-γ) and its receptor. The level of expression of IFN-γ was compared with that in tissue specimens of delayed-type hypersensitivity (DTH) reactions of the skin and of chronic tonsillitis. Furthermore, the percentage of T-lymphocytes which stained positive for IFN-γ was determined using double staining techniques. IFN-γ and its receptor were detected in all patients with RA and in 7/8 and 3/8, respectively, of patients with OA. Expression of IFN-γ (P < 0.02) and IFN-γ receptor (P < 0.01) in synovial tissue of patients with RA was more abundant compared with that in patients with OA. Although IFN-γ could be detected in RA synovial tissue, the level of expression was less when compared with DTH reactions of the skin and tonsillitis. The percentage of CD3+ cells being positive for IFN-γ was ~1% in RA, whereas in DTH reactions of the skin it was >90% and in tonsillitis ~30%. We conclude that the presence of IFN-γ and its receptor in RA synovial tissue suggests a role for this cytokine in the ongoing immunological reaction of the inflamed joint.

KEY WORDS: Interferon-gamma, Interferon-gamma receptor, T-lymphocytes, Rheumatoid arthritis, Osteoarthritis.

T-CELLS are thought to be important in the pathogenesis of rheumatoid arthritis (RA). Evidence for this hypothesis has been derived from studies demonstrating an association of the human leucocyte antigen (HLA)-DR4 with disease susceptibility and outcome [1], the presence of large numbers of activated T-lymphocytes in affected joints [2, 3], similarities in the cellular infiltrate in delayed-type hypersensitivity (DTH) reactions of the skin and RA synovial tissue [4] and intervention studies directed against T-cells that were effective in the treatment of RA [5-8].

In sharp contrast with the hypothesis suggesting an active role for T-cells in RA, data concerning the presence of T-cell-derived cytokines, such as interleukin (IL)-2, -3, -4 and interferon-gamma (IFN-γ), in synovial tissue are conflicting. Despite a few negative reports [9, 10], several groups have been able to detect messenger ribonucleic acid (mRNA) for at least one of the above-mentioned cytokines [10-14]. Evidence for the presence of the actual protein products is less convincing. Only low amounts of IFN-γ and IL-2 protein have been detected in the synovial fluids of patients with RA [15-17]. Such data are difficult to interpret because the actual amount of a particular cytokine is the result of production, uptake and degradation. For this reason direct immunohistological detection of T-cell cytokines in the synovial membrane is of interest. Immunoreactive IL-2 may be present, since in one report up to 52% of cells stained positive for this cytokine [18]. However, in another study IL-2 was not, or hardly, detectable in the rheumatoid synovial membrane [19]. In the latter study the level of IFN-γ was found to be low. The reported difficulty in detecting T-cell cytokines in RA has questioned the role of T-cells in the inflammatory process of this disease [20]. The aim of the present study is to specify further the presence of cytokine-producing T-cells in RA.

The hypothesis of this study is that T-cells do play a role in RA and that, therefore, the expression of T-cell cytokines within the synovial micro-environment is to be expected. This study focuses on the synovial expression of the T-cell cytokine IFN-γ and its receptor for several reasons. The capacity of synovial-tissue-derived T-cells to produce IFN-γ following isolation and in vitro activation has been demonstrated [21]. Furthermore, during an immunological response the percentage of IFN-γ producing T-cells outnumbers the percentage of T-cells producing other cytokines [22, 23]. In the analysis aimed at RA synovial tissue, specimens of defined DTH reactions of the skin as well as tonsils were included for the following reasons: (1) to confirm the accuracy of the staining procedure; (2) because similarities in DTH reactions of the skin and the infiltrate in RA synovial tissue have supported the hypothesis that RA is a T-cell-mediated disease [4]; and (3) tonsils were incorporated to be able to compare the expression of IFN-γ in a chronic disease like RA with that in a chronic T-cell-mediated immunological...
reaction. Synovial tissue specimens of patients with osteoarthritis (OA) were studied as an example of a process where the synovitis is probably not T-cell driven.

In order to obtain an insight into the percentage of T-cells producing IFN-γ within the various tissues under study, double staining techniques were developed. Finally, the expression of IFN-γ and IFN-γ receptor was correlated with parameters of disease activity and of local inflammation of the affected joint.

MATERIALS AND METHODS

Patients and preparation of tissue specimens

Synovial tissue specimens were obtained from 11 patients with seropositive RA who fulfilled the 1987 American Rheumatism Association (ARA) criteria for RA [24] and from eight patients with OA who were diagnosed on clinical criteria and typical X-ray changes [25]. For 10 patients with RA and four patients with OA the synovial tissue was obtained by blind biopsy using a Parker-Pearson needle [26]. In the other cases synovial tissue was obtained during joint surgery. Clinical assessments included the Ritchie articular index [27], number of swollen joints (maximal 19), a visual analogue scale (VAS) (scale 0–10) for pain in all joints and a VAS (scale 0–10) for knee pain. Groups of joints, e.g. metacarpophalangeal joints, were considered as one joint. The duration of morning stiffness was registered and the severity of morning stiffness was assessed by a VAS (scale 0–10). Also, a disease activity scale (DAS) [28] was calculated. Laboratory parameters included the levels of rheumatoid factor (RF) and C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR).

Several pieces of synovium were obtained from an inflamed knee joint of each patient. The tissue samples were placed together and snap frozen in Tissue-Tek OCT (Miles Inc., Diagnostic Division, Elkhart, IN) by immersion in methylbutane (−80°C) and stored in liquid nitrogen. Cryostat sections (5 μm) were placed on adhesive glass slides (Star Frost, Knittelgläser, Germany), air dried overnight, fixed in acetone for 10 min at room temperature and stored at −80°C until required for immunohistochemical analysis. At least three synovial biopsy specimens from each patient were fixed directly in formaldehyde, embedded in paraffin and stained with haematoxylin and eosin (HE) for histological analysis.

Punch biopsies (4 mm) of DTH reactions were obtained from patients at the outpatient clinic of the Department of Dermatology 72 h after epicutaneous challenge with the relevant antigens. For diagnostic purposes these patients were tested against a broad variety of antigens. Only reactions which resulted in erythema and induration were biopsied. Human palatine tonsils were obtained from patients with recurrent tonsillitis undergoing tonsillectomy. The tissue specimens were prepared as described above.

Monoclonal antibodies

MD2, an IgGl mouse monoclonal antibody (MAb) to human IFN-γ [29], was kindly provided by Dr P. van der Meide (BPRC, Rijswijk, The Netherlands). For the detection of IFN-γ receptor the MAb R1G10 (IgM) [30] was used. Since this antibody was found in preliminary experiments to inhibit the biological activity of IFN-γ, while not competing with the cytokine for binding to its receptor, it should be considered as directed against a receptor-associated component that functions in IFN-γ signal transduction. The usefulness and specificity of MD2 [31] and R1G10 [32] for immunohistochemistry on acetone-fixed sections has been described in detail. The specificity of staining of tissue sections with the anti-IFN-γ antibody MD2 has been confirmed by blocking experiments with recombinant IFN-γ [31]. To detect T-cells the fluorescein isothiocyanate (FITC)-conjugated MAb anti-Leu 4 (IgG1, 92-0001, Becton and Dickinson, Mountain View, CA) was used. All MAbs were titrated to obtain optimal results and isotype-matched irrelevant MAbs were included as controls for non-specific interactions.

Immunohistochemical staining

Slides were allowed to warm to room temperature and air dried. Endogenous peroxidase activity was inhibited using 0.1% sodium azide and 0.3% hydrogen peroxide 30% in phosphate buffered saline (PBS). Slides were incubated overnight at 4°C with the MAb. All MAbs were diluted in PBS containing 10% normal human AB serum (NHS). After incubation with the primary antisera the slides were incubated with goat-anti-mouse Ig (total) conjugated to horseradish peroxidase (HRP) (Dakopatts P447, Glostrup, Denmark) for 30 min at room temperature, followed by an additional incubation period of 30 min at room temperature with swine-anti-goat Ig (total) conjugated to HRP (Tago, Palo Alto, CA) was used. All MAbs were titrated to obtain optimal results and isotype-matched irrelevant MAbs were included as controls for non-specific interactions.

Slides were washed with PBS. Histochemical revelation of HRP was performed with 3-aminobenzidine (P-6001, Sigma, St Louis, MO), sealed and a solution of /?-phenylene diamine (P-6001, Sigma, St Louis, MO) was used. The slides were counter-stained with Mayer's Hamalaunlosung (Merck, Darmstadt, Germany) and after washing with tap water were mounted with Kaiser's glycerol gelatin (Merck).

To detect IFN-γ in association with CD3 positive (CD3+) cells, double staining procedures were performed. Slides were incubated overnight at 4°C with MD2, followed by incubation with goat-anti-mouse Ig (total) conjugated to tetramethylrhodamine isothiocyanate (TRITC) (T5393, Sigma, St Louis, MO) for 30 min at room temperature. The slides were blocked using 20% normal mouse serum (NMS) in PBS, followed by incubation with FITC-conjugated anti-CD3 for 1 h at room temperature. In contrast to all other antibodies, which were diluted in PBS containing 10% NHS, this MAb was diluted in 5% NHS and 5% NMS. Slides were mounted with PBS/glycerol 1:9 (v/v) containing a 0.01 M solution of p-phenylenediamine (P-6001, Sigma, St Louis, MO), sealed and stored at −20°C until required for microscopical examination.
**Microscopy analysis**

HE-stained sections were coded and randomly analysed. All areas of each biopsy section were examined and histological features were scored separately by two observers, who were blind to the clinical data. Tissues were scored for the degree of infiltration with either lymphocytes, plasma cells or polymorphonuclear cells (PMNs). A score of 0 represented no infiltration, while a score of 4 represented infiltration with numerous inflammatory cells. In addition, each tissue was assigned a synovial lining hyperplasia score (0 = 1–2, 1 = 3–4, 2 = 5–6, 3 = ≥7 cell layers). The inflammation score was determined from the sum of four components: synovial lining hyperplasia and infiltration with lymphocytes, plasma cells and PMNs. Staining for IFN-γ and IFN-γ receptor was randomly scored by two observers, who were blind to the clinical data, on a five-point scale: 0 represented no staining, while a score of 4 was given to the patients with the most abundant staining. For the scoring all areas of each biopsy section were taken into account. The suitability and reproducibility of both scoring systems have been reported previously [33]. In double label experiments co-expression of IFN-γ and CD3 was determined by counting at least 100 CD3+ cells.

**Statistical analysis**

The Mann–Whitney U test was used to determine whether there was a significant difference in the level of expression of IFN-γ and its receptor between patients with RA and OA. Spearman rank correlation coefficients were calculated to determine whether there was a significant correlation between the level of expression of IFN-γ and IFN-γ receptor with the histological inflammation score of the joint and with parameters of disease activity.

**RESULTS**

**Clinical features**

Clinical data of the patients included in this study are presented in Table I.

**Detection of IFN-γ, IFN-γ receptor and CD3+ cells in synovial tissue**

IFN-γ positive cells were detected in synovial tissue of all patients with RA (Table I and Figs 1–4). Staining

**TABLE 1**

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Disease duration (yr)</th>
<th>Medication</th>
<th>DAS</th>
<th>Cell count*</th>
<th>IFN-γ</th>
<th>IFN-γ receptor</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RA</td>
<td>M</td>
<td>48</td>
<td>D-penicillamine</td>
<td>4.76</td>
<td>2.2</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>RA</td>
<td>F</td>
<td>5</td>
<td>Diclofenac</td>
<td>2.95</td>
<td>12.4</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>RA</td>
<td>F</td>
<td>19</td>
<td>Cyclophosphamide, prednisone</td>
<td>2.85</td>
<td>2.0</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>RA</td>
<td>F</td>
<td>10</td>
<td>Prednisone, diclofenac</td>
<td>5.22</td>
<td>8.7</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>RA</td>
<td>F</td>
<td>4</td>
<td>Sulphasalazine, naproxen</td>
<td>5.62</td>
<td>ND</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>RA</td>
<td>M</td>
<td>3</td>
<td>Cyclosporin, ibuprofen</td>
<td>4.82</td>
<td>17.0</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>RA</td>
<td>M</td>
<td>6</td>
<td>Naproxen</td>
<td>3.87</td>
<td>13.6</td>
<td>1</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>RA</td>
<td>F</td>
<td>13</td>
<td>Hydroxychloroquine, indomethacin</td>
<td>4.73</td>
<td>8.0</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>RA</td>
<td>F</td>
<td>8</td>
<td>Sulphasalazine, prednisone</td>
<td>5.17</td>
<td>9.9</td>
<td>4</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>RA</td>
<td>F</td>
<td>3</td>
<td>Sulphasalazine, prednisone, diclofenac, paracetamol</td>
<td>4.18</td>
<td>22.5</td>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>RA</td>
<td>F</td>
<td>8</td>
<td>Prednisone</td>
<td>4.09</td>
<td>ND</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>OA</td>
<td>F</td>
<td>16</td>
<td>—</td>
<td>2.93</td>
<td>1.3</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>OA</td>
<td>F</td>
<td>1</td>
<td>Ibuprofen</td>
<td>1.63</td>
<td>ND</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>14</td>
<td>OA</td>
<td>F</td>
<td>6</td>
<td>—</td>
<td>2.16</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>OA</td>
<td>F</td>
<td>1</td>
<td>—</td>
<td>2.46</td>
<td>ND</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>OA</td>
<td>F</td>
<td>14</td>
<td>Paracetamol</td>
<td>2.56</td>
<td>0.3</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>OA</td>
<td>F</td>
<td>10</td>
<td>Indocid</td>
<td>3.98</td>
<td>4.3</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>OA</td>
<td>F</td>
<td>1</td>
<td>Indocid</td>
<td>4.35</td>
<td>8.5</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>19</td>
<td>OA</td>
<td>F</td>
<td>5</td>
<td>—</td>
<td>1.78</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

*Number of leucocytes × 10⁶/mm³ synovial fluid of the biopsied joint.

DAS, disease activity score; IFN-γ, interferon-gamma; RA, rheumatoid arthritis; OA, osteoarthritis.
Fig. 2.—Localization of IFN-γ in RA synovial tissue, tonsils and DTH reactions of the skin. IFN-γ was localized using immunoperoxidase-based
techniques. (A) Rheumatoid synovial tissue: expression of IFN-γ (overview, original magnification ×250). (B) Rheumatoid synovial tissue:
negative control (original magnification ×250). (C) Rheumatoid synovial tissue: mononuclear cell infiltrate, demonstrating faint expression of
IFN-γ (original magnification ×630). (D) Rheumatoid synovial tissue: mononuclear cell infiltrate, demonstrating exceptionally intense staining
for IFN-γ (original magnification ×400). (E) Human palatine tonsil demonstrating IFN-γ expression in the interfollicular T-cell zone (original
magnification ×400). (F) Mononuclear infiltrate in DTH reaction of the skin demonstrating numerous IFN-γ positive cells (original
magnification ×400).
FIG. 3.—Expression of IFN-γ and its receptor in the synovial lining of a patient with RA. IFN-γ and IFN-γ receptor were localized using immunoperoxidase-based techniques. (A) Localization of IFN-γ positive cells in the synovial lining (original magnification ×400). (B) IFN-γ receptor expression in the synovial lining (original magnification ×400). (C) Negative control for IFN-γ receptor (original magnification ×400).

For IFN-γ could be detected in association with various structures, especially with blood vessels and the synovial lining. In four patients up to 60% of small blood vessels stained positive for IFN-γ. However, this was not a general phenomenon, since in five RA patients almost no vessels stained positive for this cytokine. Although in a few patients some parts of the lining showed intense staining for IFN-γ, in none of the patients did the percentage of IFN-γ positive cells in the synovial lining exceed 35%. Only a few IFN-γ positive cells were observed in the mononuclear infiltrates in the synovium of this specific group of patients. IFN-γ positive cells were also found in synovial tissue specimens of seven of the eight patients studied with OA. The tissue distribution was similar in patients with OA and RA. Nevertheless, the expression of IFN-γ was higher in patients with RA compared with patients with OA (P < 0.02, Mann–Whitney U test).

To determine whether the presence of IFN-γ could be of functional relevance and whether co-distribution of IFN-γ and its receptor occurred, staining for IFN-γ receptor was performed. IFN-γ receptor positive cells were detected in all patients with RA and in three out of eight patients with OA (Table I and Figs 1 and 3). Staining was most intense in the synovial lining. Faint staining was observed in association with the mononuclear infiltrates. Two patients with RA showed weak staining of blood vessels. The distribution pattern for IFN-γ receptor was similar for patients with RA and OA. Although in some patients some parts of the lining showed almost identical staining for IFN-γ and its receptor the distribution pattern was not similar. For IFN-γ receptor a statistically significant (P < 0.01, Mann–Whitney U test) higher expression was found in synovial tissue of patients with RA compared with OA.

Detection of IFN-γ and its receptor in tonsils and DTH reactions of the skin

In tonsils the most prominent staining for IFN-γ was detected in the interfollicular T-cell zones (Fig. 2E). Also, intense staining of small vessels was observed. Only occasionally were positive cells observed in the germinal centres and mantle zone. The cells of the basal layer of the stratified squamous epithelium of the tonsillar capsule showed faint staining for IFN-γ. These cells of the basal layer showed very intense staining for IFN-γ receptor. The germinal centres, mantle zones, extrafollicular areas and vessels showed only faint staining for IFN-γ receptor.

In the mononuclear infiltrates of the DTH reactions of the skin the majority of cells stained positive for IFN-γ (Fig. 2F). The cells of the basal layer of the stratified squamous epithelium of the dermis showed weak staining for IFN-γ. Positive staining for IFN-γ receptor was found in association with the basal layer of the epithelium of the dermis; faint staining was observed in association with the mononuclear cell infiltrates.

Double staining for IFN-γ and CD3

Because T-cells are generally thought to be the main producers of IFN-γ, double staining procedures were
developed (Fig. 4). Synovial specimens of four patients with RA (patients 1, 5, 8 and 9) were stained for IFN-γ and CD3 and the percentage of IFN-γ positive cells was compared with that in a tonsil (n = 1) and in DTH reactions of the skin (n = 3). In all four tissue specimens of RA patients tested, IFN-γ positive T-cells could be detected, but the percentage of CD3 cells which stained positive for IFN-γ did not exceed 1%. In the inter-
follicular T-cell zones of the tonsils this percentage was around 30% and in DTH reactions of the skin the percentage of CD3 cells which stained positive for IFN-γ exceeded 90%. This analysis also revealed that the vast majority of cells in the rheumatoid synovium staining positive for IFN-γ were non-T-cells. In the interfollicular T-cell zone of the tonsil around 50% of the IFN-γ positive cells co-expressed the CD3 antigen, whereas this was more than 90% in DTH reactions of the skin.

Comparison of IFN-γ and IFN-γ receptor expression with clinical features

Spearman rank correlation coefficients were calculated to determine whether the level of expression of IFN-γ and its receptor correlated with the histological inflammation score of the joint and with parameters of disease activity and parameters of local inflammation of the affected joint. This analysis yielded no statistically significant correlations.

DISCUSSION

The present study was designed to specify further the presence of cytokine-producing T-cells in the synovium of patients with RA. The T-cell cytokine IFN-γ and its specific receptor could be detected in synovial tissue of all RA patients studied. The level of expression of IFN-γ was higher in the synovial tissue of patients with RA compared with patients with OA, but considerably lower when compared with defined T-cell-mediated immunological reactions like the DTH reactions of the skin and inflamed tonsils. The majority of cells in the rheumatoid synovium which stained positive for IFN-γ were non-T-cells. Of the T-cell population in RA only ~1% of the cells stained positive for IFN-γ, whereas in tonsils and DTH reactions of the skin these percentages were ~30% and ~90%, respectively.

The only group that reported on the presence of IFN-γ in the synovium of patients with RA found IFN-γ among numerous cell types, but according to the authors the level of expression was rather faint in comparison with the abundance of mononuclear cells infiltrating the tissue [19]. Since the percentage of IFN-γ positive cells depends strongly upon the antibody used for immunohistochemical staining, and most antibodies against cytokines are not suitable for immunohistochemistry [34], the difficulty in detecting IFN-γ could be explained by the antibody used. The suitability of the anti-IFN-γ antibody and of the staining procedure has been documented in parallel research directed at cytokine detection in DTH reactions of the skin and tonsillitis [31]. Specimens of these tissues were included in this study. In agreement with the results of Husby and Williams, IFN-γ could be detected in association with various cell types in the synovial tissue of patients with RA [19]. Although in some patients parts of the synovium showed abundant staining for IFN-γ, in general the expression was less when compared with DTH reactions of the skin and tonsils. However, enhanced expression (P < 0.02) of IFN-γ could be observed in the synovial tissue of patients with RA compared with that of patients with OA, a disease in which T-cells are generally thought not to be responsible for the ongoing synovitis.

Double staining techniques for IFN-γ and CD3 revealed the presence of IFN-γ positive T-cells in the rheumatoid synovium, although at a low frequency. Since high numbers of IFN-γ positive T-cells could be detected in other well-defined immunological reactions, the low frequency of IFN-γ positive synovial T-cells detected in this study cannot be explained by the technique used; rather, it seems to be a characteristic of the disease process itself.

It has previously been shown that T-cell clones derived from inflamed RA synovium functionally represent the Th1 T-cell type and hence are capable of producing large quantities of IFN-γ [21]. Therefore, the difficulty in detecting IFN-γ in association with T-cells in RA synovium cannot be explained by an intrinsic inability of these T-cells to produce this cytokine. One can speculate that the difficulty in detecting IFN-γ in RA might be due to a low frequency of T-cells reacting against antigens [35] or to the chronicity of the disease process [36]. However, in DTH reactions of the skin the frequency of antigen-reactive T-cells was reported to be <1% [37] and the tonsillar tissue studied was obtained from patients with chronic inflammation. Therefore, the low frequency of IFN-γ producing T-cells in the rheumatoid joint cannot be explained by a low frequency of antigen-specific T-cells or the duration of the disease process. Recent studies suggested that this low number of IFN-γ producing synovial T-cells may relate to inhibition of T-cell activation. Mediators such as transforming growth factor-β (TGFβ) and/or IL-10, which selectively suppress T-cell function, have been observed in large quantities in the joints of RA patients [38–41]. Alternatively, the low frequency of IFN-γ positive T-cells may be due to the influence of medication on T-cell activation.

Whether this low frequency of IFN-γ positive T-cells is able to sustain a T-cell-mediated disease is not known. Of interest is the study of Stoll et al. [42], who reported a very low frequency of IFN-γ positive T-cells at the site of inflammation in a proven T-cell-mediated animal model of autoimmune disease. Therefore, it seems likely that even a few IFN-γ positive T-cells are capable of maintaining inflammation.

Another finding of this study is that the vast majority of cells in the synovium of patients with RA which stained positive for IFN-γ were non-T-cells. Immunohistochemical detection of IFN-γ in association with cell types other than T-cells has been reported previously; in the rheumatoid synovial tissue IFN-γ has been detected in association with T-cells, B-cells and macrophages [19]. Similar findings were reported for other tissues; IFN-γ was detected in association with macrophages [42], vascular endothelial cells [43], trophoblasts and decidual glandular endothelium [44], histiocytes in histiocytosis X [45, 46], stellate parenchymal cells in the brain [47] and central and peripheral neurones [48]. It is not clear whether this presence reflects actual synthesis of IFN-γ by these cells or binding of IFN-γ produced...
by T-cells to cellular IFN-γ receptors or to components of the extracellular matrix [49]. To answer this question and to complement the data on the expression of IFN-γ, staining for IFN-γ receptor was performed. This analysis revealed an increased expression of IFN-γ receptor in patients with RA compared with patients with OA (P < 0.01). This is probably due to the more pronounced inflammation in RA synovial tissue when compared with OA, because upregulation and de novo expression of IFN-γ receptor during inflammation have been reported [32]. Furthermore, it became apparent that some parts, although not all, of the synovial lining stained positive for both IFN-γ as well as IFN-γ receptor. This might indicate that the anti-IFN-γ MAb MD2, besides localizing IFN-γ producing cells, also detects receptor-bound IFN-γ. However, a reasonable number of small vessels and parts of the synovial lining were positive for IFN-γ but negative for the anti-IFN-γ receptor as detected by the antibody R1G10. Since intense expression of IFN-γ receptor on endothelium and on the synovial membranes has been reported in a study in which a polyclonal antibody was used [50], this may be explained by a somewhat lower sensitivity of the MAB used in our study.

Besides binding to IFN-γ receptor, IFN-γ has been shown to bind with high affinity to heparan sulphate proteoglycans [49], extracellular matrix components being synthesized by both endothelium [51] and synoviocytes [52]. Therefore, detection of IFN-γ bound to extracellular matrix components could be an alternative explanation for the distribution pattern of IFN-γ observed in this study.

Finally, the possibility that other cell types are involved in the production of IFN-γ should be considered. Although T-cells and natural killer cells have been most widely studied in this respect, IFN-γ production has been demonstrated in macrophages isolated from mouse spleen and bone marrow [53], rat neuronal cells [54] and purified alveolar macrophages from patients with sarcoidosis [55] as well as monocytes from normal subjects [56]. Furthermore, IFN-γ mRNA has been detected in situ in macrophages [57].

In conclusion, this study shows the presence of IFN-γ and its receptor in the synovium of patients with RA. This expression was more pronounced compared with that in patients with OA. Considering the pro-inflammatory action of IFN-γ in autoimmune processes, the expression of IFN-γ together with its receptor within the synovial membrane of patients with RA may be of pathogenic significance.

References


