Cytokine production by synovial T cells in rheumatoid arthritis

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Summary

Objective. To investigate the production of cytokines by T cells in patients with rheumatoid arthritis (RA), reactive arthritis (REA) and osteoarthritis (OA).

Methods. The lymphokines interleukin (IL)-2, IL-4, interferon gamma (IFN-γ) and tumour necrosis factor beta (TNF-β), as well as the monokines IL-1, IL-6 and TNF-α, were measured by immunoassays in sera and synovial fluid (SF) from patients with RA, REA and OA. In addition, cytokine expression was studied by immunohistochemistry in synovial membrane tissue sections from patients with RA and OA.

Results. Almost 60% of RA sera contained at least one of the cytokines investigated, though in low concentrations, whereas cytokines were generally not detectable in sera from REA and OA patients. In contrast, cytokines were found in virtually all SF; thus, the majority of SF from RA patients contained IFN-γ (median level 17 pg/ml) in addition to the monokines IL-6 (4700 pg/ml) and TNF-α (157 pg/ml). IFN-γ and IL-6 (but not TNF-α) were also frequently measured in SF from REA patients, whereas OA samples typically contained only IL-6. Immunohistochemical analysis of tissue sections from RA patients revealed lymphokine expression in 0.1–0.3% of T cells, particularly IL-2 and IFN-γ, and to a lesser extent also IL-4. Interestingly, the expression of TNF-α and IL-6 by synovial T cells was also observed. The majority of cytokine-expressing T cells were CD4-positive T-helper cells typically found in perivascular areas, whereas cytokine-producing CD8-positive T cells were found distributed throughout the synovium. As expected, in specimens from OA patients, T cells were much less abundant and expression of cytokines could not be detected.

Conclusion. These data clearly demonstrate production of cytokines by T cells in RA synovial tissue, indicating that activated T cells play a role in the pathophysiological events of RA.

Key words: T cell, Cytokines, IFN-γ, IL-4, TNF-α, Rheumatoid arthritis, Reactive arthritis, Osteoarthritis, Pathogenesis.

The aetiology and pathogenesis of rheumatoid arthritis (RA) are still unresolved. In recent years, analysis of cytokine production in RA has attracted particular interest since many cytokines are involved in the regulation of the immune and the inflammatory response [1]. Although cells of the immune system are considered to be their primary sources, several cytokines can also be produced by other cell types such as fibroblasts, endothelial or even epithelial cells. Thus, in the inflamed synovium, monocytes as well as type A and type B synoviocytes have been shown to produce large amounts of cytokines, particularly pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-1 or IL-8 [2–4]. In contrast to the general agreement on the role of highly activated macrophages and synoviocytes in the pathophysiology of RA, the involvement of T cells is unclear. RA is considered an autoimmune disease, and therefore a decisive role for T cells both in initiating and maintaining the disease
process should be assumed. However, although the majority of synovial T cells bear an activated phenotype, as indicated by expression of MHC class II antigens, only a few also express the IL-2 receptor [5, 6]. This, and the failure unambiguously to demonstrate significant production of lymphokine proteins in RA synovial tissue [3, 7–11], has led to debate about the role of T cells in RA [12–16].

In an attempt to further our understanding of the role of T lymphocytes in RA, we have analysed T-cell-derived cytokines in sera and synovial fluid (SF) from patients with RA and other rheumatic disorders by immunoassays, and have also investigated cytokine expression in synovial membranes by immunohistochemical methods using single- and double-labelling techniques. The results obtained demonstrate that a relatively small subset of activated T cells in the RA synovial membrane produce lymphokines, particularly IL-2 and interferon gamma (IFN-γ), suggesting their participation in the pathophysiological processes of RA.

**Patients and methods**

**Patients**

Synovial fluid was aspirated from knee joint effusions and blood samples drawn by venepuncture at the same time. Thirty-three paired serum/SF samples were from 27 patients with RA (female/male: 22/5; median age 59 yr, range 38–76 yr; median disease duration 5 yr, range 1–23 yr); 85% of the patients were rheumatoid factor (RF) positive. Patients with reactive arthritis (REA) and osteoarthritis (OA) served as disease controls: 26 samples were obtained from 17 REA patients (female/male: 11/7; median age 34 yr, range 14–65 yr; median disease duration 5 months, range 2–42 months) and 15 samples were obtained from 15 patients with OA (female/male 8/7; median age 60 yr, range 47–81 yr; median disease duration 3 yr, range 0.5–21 yr). RA was classified according to the 1987 revised criteria of the American College of Rheumatology [17]. REA was defined as seronegative oligoarthritis with cultural evidence of sexually transmitted disease or cultural and/or serological evidence of *Salmonella* or *Yersinia* infection. OA of the knee joints was diagnosed clinically and radiologically. The majority of patients were treated with non-steroidal anti-inflammatory drugs (diclofenac, ibuprofen or oxicams). In addition, eight RA and one REA patient received glucocorticoids (<10 mg prednisone/day), and 15 RA patients were also on slow-acting anti-rheumatic drugs (methotrexate, gold salts or chloroquine). In all patients, joints were examined for pain and swelling, and the Ritchie index was determined. In RA patients, early morning stiffness was recorded in addition. Laboratory parameters included haemoglobin, platelet count, white cell counts in peripheral blood and SF; serum iron, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and RF. All patients were seen at the outpatient clinic of the Centre for Rheumatic Diseases at Lainz Hospital and presented with effusions of the knee joints. Informed consent was obtained from all patients. Control sera from 20 healthy subjects (age range 27–58 yr) were also analysed.

**Detection of cytokines in serum and SF**

Immunoassays were employed for all cytokine measurements. Assays for IL-1, IL-2, IL-4 and IL-6 were obtained from R & D Systems (Abingdon, UK), the assay for TNF-α was from Medgenix (Fleurus, Belgium), the assay for TNF-β was from Bender MedSystems (Vienna, Austria) and the assay for IFN-γ was from Centocor (Malvern, PA, USA). All assays were carefully tested with respect to reproducibility (see below), and recovery of recombinant cytokines in spiked body fluid samples (three concentrations—low, medium and high, e.g. 30, 100 and 400 pg/ml—in three sera and SF each). Possible interference by RF was tested by analysis of samples before and after absorption of RF on IgG agarose (Sigma, St Louis, MO, USA), as described [18]. The limit of detection was defined as the minimum concentration which could be reproducibly measured, i.e. with <25% inter-assay variation. It must be noted that in all assays used, the detection limit defined in this manner was higher than that given by the manufacturers. Thus, reproducible detection limits were 10 pg/ml for IFN-γ, 20 pg/ml for TNF-β, 25 pg/ml for IL-1α and IL-1β, and 30 pg/ml for IL-2, IL-4, IL-6 and TNF-α. The specificity of the assays was assessed by competition assays in which neutralizing anti-cytokine antibodies (obtained from Endogen, Boston, MA, USA or from R & D Systems) were added to selected samples as described [19]. All positive and randomly selected negative samples were measured a second time for confirmation of results, and the mean value of the two determinations was used for statistical analysis. All cytokines were below the detection limit in sera from healthy individuals. Most of the cytokine assays were validated by participating in the Cytokine Consensus Study Group of the European Workshop for Rheumatology Research [20].

**Tissue sections**

Synovial tissue was obtained from eight patients with RA and from five patients with OA at the time of knee joint surgery, and snap-frozen in isopentane cooled with dry ice immediately after excision. Cryostat sections (4–6 µm) were air-dried for 1 day at room temperature. Sections were then fixed in ice-cold acetone for 10 min and used for immunohistochemistry.

**Detection of cytokines in synovial membranes**

For cytokine detection, affinity-purified rabbit polyclonal antibodies to IL-2, IL-4, IL-6, IFN-γ and TNF-α were used (Endogen, Boston, MA, USA). In addition, monoclonal antibodies to IL-2 (Dako), IL-4 and IL-2R (Genzyme, Boston, MA, USA) were employed. Rabbit IgG (Endogen) or isotype-matched monoclonal antibodies (Dako and Becton-Dickinson) served as controls. Sections were incubated with the primary anti-cytokine antibody for 60 min. After rinsing, endogeneous peroxi-
dase was blocked with 0.3% hydrogen peroxide in Tris-buffered saline (10 mM Tris–HCl, 140 mM NaCl, pH 7.4) for 10 min. This was followed by 30 min incubation with the second antibody, a biotinylated affinity-purified goat anti-rabbit IgG or biotinylated horse anti-mouse IgG. Then, sections were incubated with the VECTASTAIN@ABC reagent (Vector, Burlingame, CA, USA) for another 30 min. Colour was developed using diaminobenzidine (Sigma), leading to brown staining. Finally, slides were counterstained with haematoxylin or Mayer’s Hämalaun solution (both from Merck, Darmstadt, Germany).

Phenotyping of cells
Monoclonal antibodies to CD3, CD4, CD8, CD20, CD45RA and CD45RO were from Becton Dickinson, Mountain View, CA, USA; antibodies to CD68 and to HLA-DR were from Dako, Glostrup, Denmark. Isotype-matched, unrelated monoclonal antibodies served as controls. Sections were incubated with the primary antibody diluted between 1:20 and 1:100 in Tris-buffered saline for 60 min at room temperature. After rinsing, alkaline phosphatase-labelled, affinity-purified rabbit anti-mouse immunoglobulins (Dako) were applied as second antibody for 30 min, followed by incubation with the APAAP complex (Dako). Colour was developed using Fast Blue (Sigma, St Louis, MO, USA) as substrate.

Characterization of cytokine-producing cells
Double-labelling experiments were performed by first staining for cytokines, followed by immunophenotyping as described above. Thus, upon light microscopy, cytokine-expressing cells appeared brown and CD antigens stained blue. The percentage of cytokine-producing cells of a given CD subset was calculated by determining the fraction of double-stained lymphocytes among all lymphocytes counted (~20,000 cells per cytokine and patient) at 400-fold magnification.

Statistical analysis
Results of cytokine determinations were correlated with serological and clinical parameters of disease activity. The data obtained in all SF investigated were used for analysis, even though some joint aspirates (maximally two) were from the same patients at different points in time. Data were processed using the SAS package. Group data are indicated as medians and ranges. The determined in order to investigate potential correlations with T-cell-derived lymphokine (Table 1A). Summarizing the data obtained with SF, RA was characterized by the presence of TNF-α, IFN-γ and IL-6; REA samples typically contained IFN-γ and IL-6, and in OA patients only IL-6 was detected. Thus, among these three most frequently detected cytokines, TNF-α was the major discriminator between RA and the other two disorders.

Quantitative analysis. IFN-γ was the most frequently detected lymphokine. Thus, the majority of SF from RA and REA patients (72 and 85%, respectively) contained IFN-γ (>10 pg/ml) in contrast to SF from OA patients (Fig. 1). Although IFN-γ levels in REA were in general somewhat higher than in RA, this difference did not reach the level of statistical significance. However, concentrations >100 pg/ml were measured in eight RA, but in only two RA samples.

IL-2, IL-4 and TNF-β were detected much less frequently than IFN-γ. Interestingly, and in contrast to the monokines IL-6 and TNF-α (see below), SF levels of these cytokines were similar to those measured in serum. Thus, IL-2 was present in two serum/SF pairs and in two additional SF (detection limit 30 pg/ml, range 36–107 pg/ml), and IL-4 was detected in three serum/SF pairs and one additional SF (detection limit 30 pg/ml, range 30–149 pg/ml). Interestingly, IL-2 and IL-4 appeared to be associated with each other since five of the six IL-2-positive samples also contained IL-4 (Table 1). Finally, TNF-β was detected in nine RA patients (four SF/serum pairs, three SF, two sera) and in four REA patients (one SF/serum pair, three SF); levels were similar in both diseases (detection limit 20 pg/ml, range 23–55 pg/ml).

The monokines IL-1α, IL-1β, IL-6 and TNF-α were determined in order to investigate potential correlations with T-cell-derived cytokines (Table 1). The results were generally in line with the literature [2–4, 8, 10, 11]. Briefly, IL-1α and IL-1β (>25 pg/ml) were detected in ~30% of patients with RA (26–182 pg/ml, median 35 pg/ml), but not in the other disorders. In contrast, high levels of IL-6 (400–800,000 pg/ml, median 4500 pg/ml) were measured in virtually all SF from RA and REA patients, and also in ~70% of OA patients, although levels were significantly lower than in the other two disorders (P < 0.01). In serum, however, IL-6 (>30 pg/ml) was found in only 27% of RA patients, but not in REA and OA patients, and levels were much
Table 1. Cytokine patterns observed in (A) sera and (B) SF from RA patients. Samples are grouped according to the number of cytokines detected per sample. For comparison, the percentages* of positive samples from patients with REA and OA are shown at the bottom.

(A) Cytokine patterns in sera from RA patients

<table>
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<th>Detection limit (pg/ml)</th>
<th>IFN-γ</th>
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<th>TNF-β</th>
<th>IL-1α</th>
<th>IL-1β</th>
<th>IL-6</th>
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No cytokines were detected in the remaining 15 samples

(B) Cytokine patterns in synovial fluids from RA patients

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<th>Detection limit (pg/ml)</th>
<th>IFN-γ</th>
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Correlation of cytokines with laboratory and clinical parameters. Apart from IL-6, which in RA and REA patients correlated with ESR and CRP (data not shown), none of the cytokines determined was associated with any clinical or serological variable analysed nor with disease duration, although TNF-α levels tended to be higher in RA patients with long-standing disease. There was also no obvious correlation with (glucocorticoid or methotrexate) treatment. In consideration of the short

lower than in SF (31–86 pg/ml, median 46 pg/ml). Finally, TNF-α (>30 pg/ml) was measured in 70% of SF from RA patients (39–382 pg/ml, median 157 pg/ml), but in only four SF from REA patients ($P < 0.001$ vs RA) and in none of the OA samples.

Correlations between cytokines. In RA patients, IFN-γ levels in SF correlated with both those of TNF-α ($r = 0.31$, $P < 0.01$; Fig. 2) and IL-6 ($r = 0.32$, $P < 0.01$). TNF-α also correlated strongly with IL-6 ($r = 0.47$, $P < 0.0001$; data not shown). These associations were seen even better in those cases where consecutive samples from individual patients (six RA and six REA, respectively) were available. Thus, changes of IFN-γ, TNF-α and IL-6 generally occurred in parallel, and were accompanied by changes in parameters related to disease activity (such as ESR, CRP, platelet count and number of lymphocytes in the joint); these changes were usually more pronounced in patients with REA (Fig. 3).

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biological half-life and the primarily auto- or paracrine mode of action of lymphokines, clear-cut quantitative correlations with systemic markers or general measures of disease activity were not necessarily to be expected. However, as mentioned above, associations were seen when looking at time courses of individual patients.

**Immunohistochemical detection of cytokines**

**Phenotyping of synovial membrane T cells.** Synovial membranes from seven of the eight patients with RA were heavily infiltrated with CD3-positive T cells (100–700 cells per field at ×400 magnification). In accordance with the literature, ~60–80% of T cells were CD45RO+ and 40–60% expressed HLA-DR [5, 6, 13, 14]. The ratio of CD4:CD8 cells ranged from 2 to 2.5. Synovial membranes from four of the five OA patients contained only a few T cells. In contrast, in sections from the fifth OA patient, more pronounced T-cell infiltrates were present; however, clusters were much smaller than those seen in RA patients (10–100 cells per field). The CD4:CD8 ratio in OA patients was comparable to that seen in patients with RA.

**Cytokine expression in synovial membranes.** In Fig. 4, single stainings for the cytokines IFN-γ, IL-2 and IL-4 are shown in tissue sections from a patient with RA. The three lymphokines were typically produced by cells infiltrating the synovium and were not found within the lining layer. These lymphokine-expressing cells tended to occur in small clusters and were particularly found in perivascular locations in close proximity to endothelial cells. In contrast, IL-6 and TNF-α were found throughout the synovium, including the lining layer, as described ([3, 21, 22]; data not shown). Although some sections from OA patients contained T-cell infiltrates, neither cytokines nor expression of IL-2R could be detected (not shown).

To determine the relative frequency (i.e. percentage) and to characterize phenotypically cytokine-producing T cells, double stainings were performed using monoclonal antibodies to CD3, CD4 and CD8. Pronounced expression of cytokines was seen in only ~0.1–0.3% of all T cells investigated; however, within some T-cell aggregates, cytokines could be detected in up to 3% of the cells. Although cytokine-expressing cells stained relatively weakly for CD antigens [presumably because the anti-cytokine antibody (applied first) interfered with binding of the anti-CD antibody], double-labelled cells can be seen in Fig. 5 where representative results obtained for IFN-γ, IL-2 and IL-4 are shown. In general, lymphokine-expressing cells were CD3 positive (or found within clusters of CD3-positive cells), belonged primarily to the CD4 population, and were typically seen in perivascular areas and in the more superficial layers of the synovial membrane. CD8-positive cells expressing cytokines were also detected, but were more diffusely distributed, occurring also in the sublining zone either within small clusters or even as single cells. As expected, IL-6 and TNF-α were produced mainly by CD68-positive cells; nevertheless, both cytokines also co-localized with CD3-positive T cells (Fig. 6).

Although due to the heterogeneity of tissue sections exact quantification of immunohistochemical data is difficult, analysis of double-stained sections revealed IL-2 to be the most frequently detected lymphokine (at an average of ~70 double-labelled cells/20 000 cells analysed/patient), followed by IFN-γ (45 cells/patient) and IL-4 (25 cells/patient), which was definitely detectable in all RA patients investigated even though in one patient as few as 11 IL-4-positive cells could be detected. The average ratio of IFN-γ:IL-4-producing cells was ~2:1, but varied considerably between individual patients, ranging between 5:1 and 1:1. Remarkably, T-cell-specific expression of TNF-α was comparable to that of IL-2, whereas IL-6 expression was similar to that of IFN-γ. Thus, the ratio of TNF-α:IL-6-producing T cells was comparable to the ratio found for IFN-γ and IL-4.

**Discussion**

The involvement of T cells in the pathophysiology of RA has been heavily disputed in the past decade [12–16]. Although it is well established that the rheumatoid synovium is infiltrated with T cells showing an activated phenotype (as indicated by the expression of HLA class II molecules and other activation markers), the majority of these cells appear to be in a state of unresponsiveness since they lack expression of the IL-2 receptor [5, 6]. Moreover, data on the presence of lymphokines in RA synovial tissue and fluid are controversial: even though mRNA for T-cell-specific cytokines has been detected by some investigators, conclusive evidence for expression of cytokine proteins by synovial T cells is still scarce [3, 7–11, 22–25]. The data presented here now unambiguously demonstrate cytokine production by T cells in the synovium of patients with RA. Thus, apart from detecting the Th1 cytokine IFN-γ in the majority of SF, we have been able to demonstrate directly expression of cytokine proteins by synovial membrane T cells using histochemical methods. In contrast, in T-cell infiltrates from OA patients, cytokine expression was generally not observed nor could lymphokines be detected in their SF. Interestingly, cytokine expression was not only seen in CD4-positive T-helper cells, but with similar (relative) frequency also in the CD8-positive population. With RA being a MHC II-associated disease, a pathophysiological involvement of CD8 cells has been largely neglected so far, although these cells might play an important regulatory role [26]. Furthermore, one should also bear in mind that CD8 cells are activated by endogenous antigens presented by MHC I molecules which may include both pathogen-derived and ‘true’ self structures.

Compared to RA, the role of T cells in REA is less controversial [27]. Therefore, it was somewhat surprising to find SF levels of IFN-γ in REA and RA to be similar. This is in line with observations reported by Simon et al. [28], who investigated cytokine mRNA expression in synovial tissue, and suggests that both disorders bear substantial similarities as far as Th1 cells
are concerned. Although relatively low when compared to TNF-\(\alpha\), the levels of IFN-\(\gamma\) in SF were comparable to those observed in peripheral blood during acute transplant rejection episodes or acute viral infections [29]. Given that IFN-\(\gamma\) is a potent activator of macrophages and (together with other stimuli) can induce or enhance TNF-\(\alpha\) production [30–32], one might also have expected high TNF-\(\alpha\) levels in REA; however, this was not the case. Hence, IFN-\(\gamma\) alone cannot be held responsible for the chronic overproduction of TNF-\(\alpha\) in RA, although the observed correlation between IFN-\(\gamma\) and TNF-\(\alpha\) levels is suggestive of a mutual relationship between these two cytokines. It has been suggested by several authors that activation of Th2 cells, and thus production of anti-inflammatory cytokines such as IL-4, may be deficient in RA and other autoimmune diseases [28, 33, 34]. This assumption is primarily based on \textit{in vitro} cytokine patterns of synovial T cells [35–37] and supported by recent observations in animal models [38, 39]. Our histochemical data may also point in this direction since fewer cells stained positive for IL-4 than for IL-2 or IFN-\(\gamma\), even though IL-4-expressing T cells were definitely seen in all RA patients investigated. Determination of IL-4 in SF, on the other hand, did not provide further information since, in contrast to IFN-\(\gamma\), this cytokine (as well as IL-2 and TNF-\(\beta\)) was only rarely detected, most likely due to the lower sensitivity of the immunoassays used. In any case, cytokine data must be interpreted with caution since it would probably be too simplistic a view to explain a complex
autoimmune disease like RA solely on the basis of a Th1–Th2 imbalance.

In the light of the apparent pivotal importance of TNF-α and IL-1 and their production by mesenchymal and myelomonocytic cells of the pannus [2–4], it has been argued that the pannus was an autonomous tissue with macrophage-like cells being central to the pathogenic events, and that T cells entered the synovial membrane as ‘innocent bystanders’ rather than as cells specifically involved in the inflammatory process [12, 40–42]. According to this assumption, T-cell activation would merely reflect a secondary phenomenon or just a reaction of T cells to the ongoing destructive process. Moreover, the numbers of synovial T cells secreting cytokines could be regarded as being far too low to exert significant effects. However, several lines of evidence suggest that T cells play an important role in the pathogenesis of RA. (i) It is well established that small numbers of antigen-specific T cells are sufficient to drive inflammatory (immune and autoimmune) processes [43, 44]. For example, the frequencies of antigen-specific T cells seen in the cerebrospinal fluid of patients with multiple sclerosis or in skin lesions of patients suffering from leprosy or allergic contact dermatitis are well below 1%, and thus of the same order of magnitude as the frequency of cytokine-expressing T cells reported here [45–47]. (ii) RA is a disease strongly associated with certain MHC class II alleles, suggesting involvement of certain T-cell receptors [13, 15, 48]. (iii) T cells are already seen in the synovium at very early stages of RA and with progressing disease there appears to be little change of the synovium’s immunohistological features [49]; furthermore, cytokine-expressing (naive) T cells have been observed in the peripheral blood of RA patients [50]. (iv) Oligoclonality of synovial T-cell populations has been repeatedly described [51–54] and germinal centre-like structures have been detected in RA synovial tissue [55]. (v) Treatment directed towards T cells, such as therapy with cyclosporin A, can lead to significant clinical effects in RA [56] and anti-CD4 monoclonal antibody therapy has been shown to lead to a decrease of monokines as well as to reduction of the inflammatory synovitis, including synovial lining cell hyperplasia [57–59], although the clinical benefits obtained are debatable [60, 61]. (vi) Furthermore, evidence has been provided recently
Fig. 6. Immunohistochemical characterization of synovial cells expressing TNF-α (A) and IL-6 (B) in synovial tissue sections from a patient with RA. For technical details, see Fig. 5. Although the majority of TNF-α- and IL-6-expressing cells were CD68 positive, some CD3-positive T cells also stained positive for these two cytokines. Similar to IL-2-expressing cells, such T cells typically were seen within relatively large clusters of T cells.

that T cells stimulated by IL-2 or IL-15 in an antigen-independent manner can induce TNF-α production by monocytes [62, 63], and that T cells activated via the TCR complex are also able to modulate IL-10 expression [64]. Interestingly, T-cell membrane TNF-α was identified as one of the contact-mediated signals required for the regulation of IL-10 production. Our finding of TNF-α expression by synovial T cells suggests that this regulatory circuit might be of relevance in RA. (vii) Most inducible animal models of RA (and other autoimmune diseases too) appear to be T-cell dependent. Nevertheless, even in collagen arthritis, substantial amounts of lymphokines are found only in the early stage, whereas monokines largely predominate at late stages when anti-T-cell therapy becomes less effective [65, 66]. Finally, a novel animal model has been described recently in which spontaneously developing RA-like inflammatory joint disease was observed in a mouse strain obtained by crossing a TCR transgenic mouse with the (diabetes-prone) NOD strain [67]. In these animals, disease development was heavily dependent upon T cells, even though joint histology showed an abundance of cells of the myeloid lineage and a cytokine profile similar to human RA.

Taken together, these observations allow the conclusion to be drawn that a relatively small number of cytokine-secreting T cells may indeed play a substantial role in the inflammatory synovitis of RA both by initiating and maintaining the disease process. Even though the majority of synovial T cells appear to be in a state of hyporesponsiveness, which may be due to long-term exposure to endogeneously produced TNF-α and/or IL-10 [68–70], the continuous presence of relatively few activated (Th1-like) cells could still be sufficient to drive the autoimmune process. This does not exclude that with progressing disease the synoviocytes/monocytes become more and more autonomous, requiring less support by T cells. In therapeutic terms, this would imply that interference with both cellular compartments is required for successful treatment of RA. Such a conclusion is supported by recent evidence of improved efficacy of combination therapies in experimental models of arthritis as well as in human RA [71–73]. In this context, it may be interesting to note that treatment with depleting anti-CD4 antibody apparently does not affect IFN-γ-secreting Th1 cells [74], which may (partially) explain the modest benefits achieved in most clinical anti-CD4 trials [60, 61, 73]. In any case, the presence of cytokine-expressing T cells in RA synovium is suggestive of antigen-specific activation, and the identification of relevant antigens will remain a challenging task for future investigations.

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