Expression of interferon β (IFN-β) in synovial tissue from rheumatoid arthritis patients compared to osteoarthritis and reactive arthritis patients.

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Key words
Rheumatoid arthritis; interferon beta; expression; synovial tissue; cytokines
Abstract

Objectives. IFN-β may have immunomodulatory effects in rheumatoid arthritis (RA). In addition, IFN-β has been shown to be chondroprotective in collagen induced arthritis (CIA) in mice. Increased IFN-β production in RA synovium could represent a reactive attempt to inhibit inflammation. The aim of this study was to determine the expression of IFN-β in synovial tissue of patients with RA, osteoarthritis (OA), and reactive arthritis (ReA).

Methods. Synovial biopsy specimens were obtained by arthroscopy from RA patients and disease controls for immunohistologic analysis using a monoclonal antibody specific for IFN-β. Bound antibody was detected according to an immunoperoxidase method. Stained sections were evaluated by computer-assisted image analysis. Furthermore, double stainings were performed with antibodies to detect CD55 positive fibroblast-like synoviocytes (FLS), CD68 positive macrophages, and CD83 positive dendritic cells (DC) co-expressing IFN-β.

Results. IFN-β protein was abundantly expressed in the synovium of RA patients. Digital image analysis using a standardized program revealed a statistically significant increase in the mean integrated optical density for IFN-β expression in RA synovial tissue compared to disease controls. The specific upregulation of IFN-β expression was also observed when the results were controlled for cell numbers. Phenotypic analysis revealed that especially FLS, but also macrophages and DCs may express IFN-β in RA synovial tissue.

Conclusions. The increased expression of IFN-β in RA synovium suggests activation of an immunomodulatory mechanism that could inhibit synovial inflammation.
Introduction
Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting synovial tissue in multiple joints. The rheumatoid synovium is characterised by infiltration of inflammatory cells like macrophages and fibroblast-like synoviocytes (FLS), which play a crucial role as effector cells in RA.

Interferon-β (IFN-β) is emerging as a molecule with a possible beneficial effect on arthritis activity [1-3]. Interferons are a family of naturally secreted proteins with immunomodulatory functions [4]. IFN-β, a type I IFN, has mainly anti-inflammatory properties and can exert a variety of immunological effects [5;6]. An additional reason why IFN-β could be of interest in RA, is its inhibitory effect on osteoclastogenesis. IFN-β plays an important role in bone homeostasis by inhibiting c-Fos induction required for osteoclastogenesis [7;8] Consistent with these studies recent work has shown that daily subcutaneous treatment with IFN-β inhibits the development of erosive disease in an animal model of RA [9].

So far, little is known about the endogeneous production of IFN-β in synovium of RA patients. One report suggested high IFN-β expression in synovial tissue of 6 RA patients compared to patients with osteoarthritis (OA), but the sample size did not allow statistical analysis [10]. In addition, double staining for phenotypic analysis was not performed. This motivated us to examine protein expression of IFN-β in the synovium of a larger number of RA patients in comparison with synovial tissue from OA and reactive arthritis (ReA) patients as disease controls. In addition, we performed phenotypic analysis to determine the expression of IFN-β by FLS, macrophages and dendritic cells (DC) in rheumatoid synovium.
Patients and Methods
Seventeen RA patients underwent synovial biopsy of an inflamed knee joint. All RA patients met the criteria of the American College of Rheumatology (ACR) for the diagnosis of RA [11]. In addition, synovial biopsies were obtained from 9 inflammatory OA and 6 ReA patients. Clinical data of these patients are presented in Table 1. At the time of inclusion all RA, OA and ReA patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs), 2 RA patients received disease modifying anti-rheumatic drugs (DMARDs) and 1 RA patient was treated with corticosteroids. None of the OA and ReA patients were treated with DMARDs or corticosteroids. All patients gave written informed consent and the study protocol was approved by the Medical Ethics Committee.

Specimen collection
Biopsy specimens were taken from the knee under local anaesthesia using arthroscopy. Arthroscopies, tissue sampling and storage were performed as described previously [12].

Immunohistochemical analysis
Sections were stained with a monoclonal antibody (mAb) against IFN-β (PBL, Florence, Italy). Staining was performed according to a 3-step immunoperoxidase method as previously described [13].

Digital image analysis
The slides were evaluated by digital image analysis. All sections were coded and analysed in a random order by an independent observer who was blinded for the clinical data as described previously [14].

Double staining
To investigate which percentage of FLS, macrophages and DCs express IFN-β in RA synovium, we performed double staining using light microscopy with the following mAbs: anti-CD68 (Dako, Glostrup, Denmark), anti-CD55 FITC (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), anti-CD83 FITC (Becton-Dickinson, San Jose, CA), and IFN-β (PBL).

Frozen sections were incubated with IFN-β (IgG1) in TBS/BSA 1% overnight at 4°C. Subsequently, sections were incubated with HRP- conjugated goat-anti-mouse in TBS/BSA 1% for 30 minutes at room temperature (RAT). Before applying biotin-tyramide for 15 minutes and streptavidine-HRP for 30 minutes, sections were incubated with 10% normal mouse serum for 15 minutes. HRP activity was detected using AEC as dye. Thereafter, anti-CD68 (IgG3) diluted in TBS/BSA 1%, was applied for 1 hour at RT in the dark and subsequently goat-anti-mouse IgG3-AP was applied for 30 minutes at RT in the dark. Fast Blue staining (Vector Laboratories, Burlingame, CA) was used to develop the AP signal.

For detection of CD55 and CD83, anti-CD55-FITC and anti-CD83-FITC, respectively, were used, followed by rabbit anti-FITC (DAKO) and swine-anti-rabbit-AP (DAKO). Fast Blue staining (Vector Laboratories) was used to develop the AP signal. For control sections, the primary antibodies were omitted or irrelevant isotype-matched mouse antibodies were applied.

Co-expression of CD68, CD55 and CD83 positive cells with IFN-β in the synovium of 6 RA patients was determined by counting positive cells. The results were noted as follows: 0-5 per cent, 6-25 per cent, 26-50 per cent, 51-75 per cent, or 76-100
percent double staining cells. All sections were analysed in a blinded manner by two independent observers.

**Statistical analysis**
The Kruskal-Wallis test was used to compare scores (expressed in interoptical density/mm² for image analysis) of IFN-β between all (RA, OA and ReA) diagnostic groups. The Mann Whitney U test was used to compare the differences between 2 groups.
Results

Clinical features
The clinical features and demographic characteristics of the patients are shown in Table 1. The mean age of patients with ReA was lower than that of patients with RA and inflammatory OA (P = 0.003). Patients from the RA and ReA group had on average a shorter disease duration than patients in the OA group.

Increased IFN-β protein expression in rheumatoid synovial tissue.
All negative controls were negative. Staining for IFN-β was found in all compartments of the synovium, but especially in the intimal lining layer. Examination of stained sections by digital image analysis using a standardized program revealed a statistically significant increase in the mean integrated optical density [14] for IFN-β expression in RA synovial tissue compared with controls (RA 1900 ± 514 vs OA 447 ± 171, and ReA 358 ± 195 ) (P = 0.04). To exclude the possibility that this difference could be explained by differences in synovial cell infiltration between RA and disease controls, we also determined IFN-β expression after correcting for cell numbers (RA 33 ± 13.2 vs OA 7 ± 4.8, and ReA 3 ± 1.8) (P = 0.02) (Fig. 1).

Expression of IFN-β by fibroblast-like synoviocytes, macrophages, and dendritic cells.
To examine which cell types may express IFN-β in rheumatoid synovium, we performed double label experiments. Staining for IFN-β was particularly found in FLS, and to a lesser extent in macrophages and DCs (Fig. 2). In RA synovium about 76-100% of the FLS, 6-25% of the macrophages, and 26-50% of the DCs were IFN-β positive.
Discussion
In the present study, the expression of IFN-β was determined in synovial tissue from patients with RA, inflammatory OA, and ReA. Digital image analysis of stained tissue sections revealed a marked increase in IFN-β protein expression in rheumatoid synovial tissue compared to disease controls. The increased expression of IFN-β could not be explained by increased cellularity in RA, since after correction for cell numbers the differences remained significant.

Phenotypic analysis showed that especially FLS, but also macrophages and DCs, in rheumatoid synovium may express IFN-β. The effector phase in RA is thought to be mediated in part by activation of FLS and macrophages, which produce a variety of proinflammatory cytokines. Mature DCs may present antigens to memory T-cells and in turn activate T-cells leading to activation of FLS and macrophages.

In light of the anti-inflammatory properties of IFN-β [1;2] we suggest that the increased expression of this cytokine by FLS and macrophages in rheumatoid synovial tissue could represent a reactive anti-inflammatory mechanism. Apparently, this anti-inflammatory response is insufficient to inhibit RA activity completely.

Since FLS, macrophages and DCs play such important roles in the initiation and persistence of inflammation, IFN-β could theoretically also promote inflammation. Moreover, a previous report suggested that IFN-β might be capable of protecting T-cells from undergoing apoptosis and that in consequence, the presence of IFN-β in RA synovium could lead to the maintenance of chronic inflammation [10]. However, we and others have shown that daily IFN-β treatment in CIA in both mice and rhesus monkeys suppresses synovial inflammation [9] [2;3]. In these animal experiments we did not observe an increase in T-cell numbers, and any potential anti-apoptotic effect did not prevent a beneficial therapeutic effect of IFN-β in vivo. Taken together, the available data suggest that the net effect of IFN-β in arthritis is anti-inflammatory. The recent observation that IFN-β may inhibit osteoclastogenesis indicates that the effects of IFN-β in RA could also be operative on the level of joint destruction [8].

The results presented here show that IFN-β is expressed in various forms of arthritis with a specific increase in protein expression in RA. Considering the previously demonstrated beneficial effects of IFN-β on synovial inflammation and bone homeostasis in vitro and in vivo, we propose that the markedly increased expression of IFN-β in RA synovium represents activation of a partially incomplete anti-inflammatory response that could reduce synovial inflammation and perhaps more importantly might inhibit bone destruction.
**Competing interests**

Dr Tak has received support from Serono for a clinical study investigating the role of interferon-β in rheumatoid arthritis patients. Dr van Holten has received a research grant from the Serono Pharmaceutical Research Institute.
Legends to Figures

Figure 1. IFN-β expression (IFN-β integrated optical density/cell) in synovium from patients with rheumatoid arthritis, inflammatory osteoarthritis, and reactive arthritis, detected by immunohistochemistry and evaluated by digital image analysis.

Figure 2. Double staining of rheumatoid arthritis synovial tissue with A, CD83 positive dendritic cells (blue) and IFN-β (red), B CD68 positive macrophages (blue) and IFN-β (red), and C CD55 positive fibroblasts like synoviocytes (blue) and IFN-β (red). Yellow arrows are pointing out examples of double staining cells, black arrows are pointing out examples of IFN-β positive cells (original magnification x 400).
Reference List


(6) Jungo F, Dayer JM, Modoux C, Hyka N, Burger D. IFN-beta inhibits the ability of T lymphocytes to induce TNF-alpha and IL-1beta production in monocytes upon direct cell-cell contact. Cytokine 2001; 14(5):272-282.


Table 1. Clinical data of patients with RA, OA and ReA who were studied for the expression of IFN-β in synovial tissue.

<table>
<thead>
<tr>
<th></th>
<th>RA patients N = 17</th>
<th>OA patients N = 9</th>
<th>ReA patients N = 6</th>
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<td>Sex, no. males/females</td>
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<td>1/8</td>
<td>3/3</td>
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<tr>
<td>Age, mean ± SD years</td>
<td>62 ± 3</td>
<td>74 ± 2</td>
<td>44 ± 6</td>
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<tr>
<td>Disease duration, mean ± SD months</td>
<td>7 ± 3</td>
<td>98 ± 73</td>
<td>19 ± 12</td>
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<td>RF, no. positive/negative</td>
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<td>0/6</td>
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<tr>
<td>CRP, mg/liter</td>
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<td>15 ± 6</td>
<td>12 ± 5</td>
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<td>Erosions, no. with/without</td>
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<td>0/6</td>
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Figure 1.