CONCISE REPORT

Expression of interferon β in synovial tissue from patients with rheumatoid arthritis: comparison with patients with osteoarthritis and reactive arthritis

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Ann Rheum Dis 2005;64:1780–1782. doi: 10.1136/ard.2005.040477

Background: IFN β may have immunomodulatory effects in rheumatoid arthritis (RA) and its increased production in RA synovium may be a reactive attempt to inhibit inflammation. **Objective:** To determine the expression of IFN β in the synovial tissue of patients with RA, osteoarthritis, and reactive arthritis.

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

Results: IFN β protein was abundantly expressed in the synovium of patients with RA. Digital image analysis showed a significant increase in the mean integrated optical density for IFN β expression in RA synovial tissue compared with disease controls. Specific up regulation of IFN β expression was also seen when the results were controlled for cell numbers. Phenotypic analysis showed that FLS, especially, 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.

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 have 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 might be of interest in RA, is its inhibitory effect on osteoclastogenesis. IFN β has 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 endogenous production of IFN β in the synovium of patients with RA. One report suggested high IFN β expression in the synovial tissue of six

patients with RA compared with 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 compare protein expression of IFN β in the synovium of a larger number of patients with RA with its expression in synovial tissue from patients with OA and reactive arthritis (ReA) as disease controls. In addition, we performed phenotypic analysis to determine the expression of IFN β by FLS, macrophages, and dendritic cells (DCs) in rheumatoid synovium.

PATIENTS AND METHODS

Seventeen patients with RA underwent synovial biopsy of an inflamed knee joint. All patients with RA met the criteria of the American College of Rheumatology for the diagnosis of RA.¹¹ In addition, synovial biopsy specimens were obtained from nine patients with inflammatory OA and six with ReA. Table 1 presents the clinical data of these patients. At the time of inclusion all patients with RA, OA, and ReA were treated with non-steroidal anti-inflammatory drugs, two patients with RA received disease modifying antirheumatic drugs, and one patient with RA was treated with corticosteroids. None of the patients with OA and ReA 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 against IFN β (PBL, Florence, Italy). Staining was performed according to a three 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 unaware of the clinical data as described previously.¹⁴

Double staining

To investigate the percentages of FLS, macrophages, and DCs that express IFN β in RA synovium, we performed double staining using light microscopy with the following

Abbreviations: AP, alkaline phosphatase; BSA, bovine serum albumin; FLS, fibroblast-like synoviocytes; HRP, horseradish peroxidase; IFN β , interferon β ; OA, osteoarthritis; RA, rheumatoid arthritis; ReA, reactive arthritis; TBS, Tris buffered saline

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

Clinical data	Patients with RA (n = 17)	Patients with OA (n = 9)	Patients with ReA (n = 6)
Sex, men/women (n)	6/11	1/8	3/3
Age (years), mean (SD)	62 (3)	74 (2)	44 (6)
Disease duration (months), mean (SD)	7 (3)	98 (73)	19 (12)
RF, positive/negative (n)	11/6	0/9	0/6
CRP (mg/l), mean (SD)	59 (12)	15 (6)	12 (5)
Erosions, with/without (n)	4/13	0/9	0/6

monoclonal antibodies: 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 Tris buffered saline (TBS)/bovine serum albumin (BSA) 1% overnight at 4°C. Subsequently, sections were incubated with horseradish peroxidase (HRP) conjugated goat-antimouse in TBS/BSA 1% for 30 minutes at room temperature. Before applying biotin-tyramide for 15 minutes and streptavidin-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 room temperature in the dark and, subsequently, goat-antimouse IgG3-alkaline phosphatase (AP) was applied for 30 minutes at room temperature 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-antirabbit-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.

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

Figure 1 IFN β expression in synovium from 17 patients with RA, nine with inflammatory OA, and six with ReA, detected by immunohistochemistry and evaluated by digital image analysis.

Statistical analysis

The Kruskal-Wallis test was used to compare scores (expressed in integrated optical 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 two groups.

RESULTS

Clinical features

Table 1 shows the clinical features and demographic characteristics of the patients. 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 had no positive staining. 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 standardised program showed a significant increase in the mean (SEM) integrated optical density¹⁴ for IFN β expression in RA synovial tissue compared with controls (RA 1900 (514) ν OA 447 (171), and ν ReA 358 (195), $p = 0.04$). To exclude the possibility that this difference might be explained by differences in synovial cell infiltration between RA and disease controls, we also determined IFN β expression (mean (SEM)) after correcting for cell numbers (RA 33 (13.2) ν OA 7 (4.8), and ν ReA 3 (1.8), $p = 0.02$) (fig 1).

Expression of IFN β by FLS, macrophages, and DCs

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 this 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 showed a marked increase in IFN β protein expression in rheumatoid synovial tissue compared with disease controls. The increased expression of IFN β could not be explained by increased cellularity in RA, because after correction for cell numbers the differences remained significant.

Phenotypic analysis showed that FLS, especially, 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 may represent a reactive anti-inflammatory mechanism. Apparently, this anti-inflammatory response is insufficient to inhibit RA activity completely.

Because FLS, macrophages, and DCs have such important roles in the initiation and persistence of inflammation, IFN β might 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 might lead to the maintenance of chronic inflammation.¹⁰ However,

Figure 2 Double staining of RA 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 point to examples of double staining cells, black arrows point to examples of IFN β positive cells (original magnification $\times 400$).

we and others have shown that daily IFN β treatment in collagen induced arthritis in both mice and rhesus monkeys suppresses synovial inflammation.^{2,3,9} In these animal experiments we did not see 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 might also affect 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 might reduce synovial inflammation and, perhaps more importantly, might inhibit bone destruction.

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Competing interests: Dr Tak has received support from Serono for a clinical study investigating the role of interferon β in patient with rheumatoid arthritis. Dr van Holten has received a research grant from the Serono Pharmaceutical Research Institute.

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Accepted 2 May 2005

Published Online First 5 May 2005

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