Differential in vitro effects of IL-4, IL-10, and IL-13 on proinflammatory cytokine production and fibroblast proliferation in rheumatoid synovium

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Abstract The purpose of this study was to compare the potential of interleukin-4 (IL-4), IL-10, and IL-13 to interrupt two major inflammatory pathways in rheumatoid arthritis (RA), i.e., overexpression of proinflammatory cytokines and cytokine-mediated fibroblast growth. IL-4, IL-10, and IL-13 were all able to significantly inhibit the production of IL-1β, tumor necrosis factor-α (TNF-α), IL-6, and IL-8 by freshly isolated RA synovial tissue cells; IL-10 was most effective in terms of IL-1β and TNF-α reduction. The IL-1 receptor antagonist was enhanced by IL-4 and IL-13, but only slightly enhanced by IL-10. Spontaneous interferon-γ secretion was diminished by IL-4 and IL-10 but not by IL-13. Addition of anti-IL-10 neutralizing antibody to RA synovial tissue cells resulted in a substantial increase in IL-1β and TNF-α levels, whereas neither anti-IL-4 nor anti-IL-13 antibody had a significant effect. IL-1β-stimulated proliferation of RA synovial fibroblast cell lines was inhibited by IL-4 and IL-13, but not by IL-10; IL-4 was over tenfold more effective than IL-13. These results suggest that IL-4, IL-10, and IL-13 all have the therapeutic potential to regulate the disease activity mediated by proinflammatory cytokines in RA, but each cytokine may have different potencies.

Key words Anti-inflammatory cytokine · IL-4 · IL-10 · IL-13 · Rheumatoid arthritis

Introduction

In the joints of active rheumatoid arthritis (RA), large amounts of proinflammatory cytokines are persistently expressed. Cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and IL-8 are produced mainly by tissue macrophages, and IL-6 is a major product from activated fibroblast-like synoviocytes [1–4]. In addition, recent studies have indicated that interferon-γ (IFN-γ), an essential mediator of Th1-type immune responses may be involved in the pathogenesis of RA [5–7], inspite of its low expression detected in the joints. These cytokines are believed to be responsible for various aspects of chronic inflammation in RA by their complex interaction. One of their major properties is to induce synovial lining cell hyperplasia, as the activation and proliferation of fibroblast cell lines isolated from RA synovium are induced by several cytokines, such as IL-1, TNF-α, transforming-growth factor, fibroblast-growth factor, and platelet-derived growth factor [8–11]. Other pathogenic events mediated by cytokine overexpression include an infiltration of blood-derived inflammatory cells, degradation of extracellular matrix components, and systemic inflammatory reactions [1]. Therefore, cytokines are the potential therapeutic targets in RA. This notion has recently been supported by reports on the clinical efficacy of anti-TNF-α antibody (Ab) and the IL-1 receptor antagonist (IL-1Ra) in the treatment of active RA patients [12, 13].

The cytokines IL-4, IL-10, IL-13 are grouped together as anti-inflammatory cytokines on the basis of their common ability to inhibit monocyte production of proinflammatory cytokines [14, 15], and they are expected to be potential therapeutic agents in certain inflammatory diseases, such as RA. IL-10 is known to be a major endogenous regulator of inflammatory cytokines produced by macrophages and T cells in RA joints [5, 16]. IL-4 has been demonstrated to inhibit both the production of cytokines in synovial tissue organ cultures [17] and the proliferation of cytokine-stimulated
fibroblasts in RA [18]. However, striking differences have been noted in their immunoregulatory properties. For example, the expression of major histocompatibility complex antigens on activated blood monocytes is increased by IL-4 and IL-13, but decreased by IL-10. On the other hand, their expression of Fc receptors for immunoglobulin G (IgG) (CD64, CD32, and CD16) is downregulated by IL-4 and IL-13, which can be prevented by IL-10 [14].

In this study, we compared the potential of IL-4, IL-10, and IL-13 to interrupt chronic inflammation in RA joints, by investigating their effects on cytokine levels produced spontaneously by synovial tissue cells and proliferative responses of cytokine-stimulated fibroblast cell lines.

Materials and methods

Cytokines and reagents

Recombinant human IL-13 (rhIL-13) and rhIL-10 were generously provided by Dr. G. Zarutski (DNAX Research Institute, Palo Alto, Calif., USA) and Dr. K.-W. Moore (DNAX Research Institute), respectively. rhIL-1β and the enzyme-linked immunosorbent assay (ELISA) kits specific for hIL-1β, hTNF-α, hIL-6, hIL-8, hIL-1RA, and hIFN-γ were kind gifts from Y. Ohmoto (Otsuka Pharmaceutical, Tokushima, Japan). The rhIL-4 was provided by Daippon Pharmaceutical (Osaka, Japan). Purified rabbit IgG anti-hIL-13 polyclonal Ab was purchased from Pepro Tech (Hoboken, NJ, USA), and purified goat IgG anti-rhIL-10 and anti-hIL-4 polyclonal Ab were purchased from R&D Systems (Minneapolis, Minn., USA). Capture and biotinylated detecting rat anti-hIL-10 monoclonal Ab (mAb) (JES3–9D7 and JES3–12G8) were purchased from PharMingen (San Diego, Calif., USA).

Isolation and culture of synovial tissue cells

Synovial tissues were obtained at the time of joint replacement or synovectomy for 19 patients with RA who satisfied the revised American College of Rheumatology (ACR) criteria [19]; serial numbers were used to identify each sample (RS1–19). Most patients were active, as they had multiple joint pain or swelling and elevated serum C-reactive protein (mean ± SD = 1.87 ± 1.40 mg/dL). Fifteen patients were positive for serum IgM rheumatoid factor, with a mean ± SD titer of 255 ± 248 IU/ml (normal range < 19 IU/ml). All patients were taking non-steroidal anti-inflammatory drugs. Sixteen patients were receiving various disease-modifying anti-rheumatic drugs and five patients were receiving 5 mg/ml of prednisolone. All patients gave informed consent. Synovial tissue cells were isolated as previously described [20]. Briefly, fragmented synovial tissues were treated with collagenase (Wako Pure Chemical, Osaka, Japan) and DNase (Sigma Chemical, St. Louis, Mo., USA) for 1 h at 37 °C. After removing tissue debris through a cell strainer, cells were washed twice with medium. The resultant single-cell suspensions were dispensed into the wells of a 24-well microtiter plate (Costar, Cambridge, Mass., USA) at a density of 2 × 10^6/ml in 2 ml of Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies, Gaithersburg, Md., USA) supplemented with 10% heat-inactivated fetal calf serum (FCS; Life Technologies), 25 mM HEPES (Life Technologies), 100 IU/ml penicillin, and 100 μg/ml streptomycin. Cells were incubated with or without 50 ng/ml of rhIL-4, rhIL-10, or rhIL-13, or 5 μg/ml of neutralizing Ab against hIL-4, hIL-10, or hIL-13 at 37 °C in a humidified atmosphere containing 5% CO₂. Supernatants were harvested 72 h later and stored at −30 °C until assayed for cytokine levels.

Preparation and proliferation assay of synovial fibroblasts

Synovial fibroblasts were obtained as previously described [21]. In brief, synovial tissue cells from RA were split weekly when primary cultures had reached confluence, and used after the third passage. The cells obtained were morphologically homogenous fibroblast-like cells. These cells were allowed to adhere to a 96-well flat bottomed microtiter plate (Costar) at a density of 1 × 10^4 per well, and then cultured in duplicate for 72 h in 100 μl of DMEM with 1% FCS and 10⁻⁸ M indomethacin (Wako Pure Chemical) containing the indicated concentrations and combinations of rhIL-1β, rhIL-4, rhIL-10, and rhIL-13. H-thymidine (Amersham, Tokyo, Japan; 1 μCi) was added to each well during the last 12 h. Adherent cells were detached by incubating with phosphate-buffered saline (PBS) containing trypsin-ethylenediaminetetraacetic acid (EDTA) for 30 min and were harvested on glass fiber filters using an automated cell harvester. Incorporated H-thymidine was measured in a liquid scintillation counter [D (Tri-Carb 2300TR; Packard, Meriden, Conn., USA) PI1].

Measurement of cytokines

IL-10 concentrations were measured by the sandwich ELISA, as described previously [22]. Briefly, samples and rhIL-10 standards were incubated in duplicate at 4 °C overnight in the ELISA plate precoated with anti-IL-10 capture mAb (JES3–9D7). After washing, plates were incubated at room temperature for 1 h with biotinylated anti-IL-10 detecting mAb (JES3–12G8). Plates were washed and incubated with avidin-peroxidase (Sigma Chemical) for 45 min. After the last washing, 2.5 μg/ml of 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical) substrate buffer was added to each well. Optical densities were determined at 405 nm. The sensitivity of this assay was approximately 10 pg/ml. IL-1β (detection limit, 20 pg/ml), TNF-α (20 pg/ml), IL-6 (20 pg/ml), IL-8 (20 pg/ml), IL-1Ra (200 pg/ml), and IFN-γ (20 pg/ml) were measured in duplicate using the ELISA systems, as described previously [23].

Detection of cytokine mRNA by reverse transcriptase-polymerase chain reaction

Freshly isolated synovial tissue cells from two RA patients were cultured for 18 h with or without 50 ng/ml of rhIL-4, rhIL-10, or rhIL-13, and 4 × 10⁶ cells were lysed in guanidinium thiocyanate buffer. Total cellular RNA was extracted using the single step method of RNA isolation [24], and poly(A⁺) RNA was separated further from total RNA dissolved in Tris/EDTA (TE) buffer by using oligo-dT latex beads (Oligo-dT30 super; Takara Shuzo, Otsu, Japan) according to the manufacturer’s instructions. Cytokine mRNA was semi-quantified by polymerase chain reaction (PCR), as described previously [25]. Briefly, cDNA was synthesized from mRNA with M-MLV reverse transcriptase (RT) (United States Biochemical, Cleveland, Ohio, USA) and oligo(dT)₁₅ primers (Promega, Madison, Wis., USA). cDNA samples were normalized to the amounts of β-actin products amplified by PCR for 30 cycles, and then were subjected to PCR amplification in a thermal cycler (Tecche PHL-3; Tecche Cambridge, Duxford, Cambridge, UK) with recombinant Taq DNA polymerase (Promega) and a pair of primers specific for IL-1β and TNF-α. Each cycle consisted of 1-min-denaturation at 94 °C, 1-min-annealing at 65 °C, and 1-min-extension at 72 °C. To ascertain that differences in cytokine mRNA expression were not due to bias in the PCR methodology, each cDNA sample was amplified by different cycle numbers as indicated. PCR products were electrophoresed on a 1.5% agarose gel and visualized by ethidium bromide staining. The reproducibility of our PCR was confirmed by repeated PCR analysis of the same samples. The sequences of oligonucleotide primers for β-actin, IL-1β, and TNF-α have been previously described [25].

In addition, the detection of IL-10 receptor transcripts in synovial fibroblast cell lines was examined by RT-PCR.