High Levels of Interleukin 13 in Rheumatoid Arthritis Sera Are Modulated by Tumor Necrosis Factor Antagonist Therapy: Association with Dendritic Cell Growth Activity

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ABSTRACT. ***Objective.*** To investigate the physiology of interleukin 13 (IL-13) in rheumatoid arthritis (RA) and the effects of tumor necrosis factor (TNF) antagonists (etanercept) on the distribution of IL-13 in patients with RA.

Methods. We measured cytokine levels in RA sera (pre/post etanercept), RA synovial fluid (SF), osteoarthritis (OA) SF, and normal human sera by ELISA. Detection of IL-13 was not influenced by rheumatoid factor, as revealed in spike recovery and isotype antibody control studies. Biologically active IL-13 in RA SF was studied using dendritic cell (DC) progenitors that develop into mature DC with IL-13 and with neutralizing antibodies to IL-13. The modulation of IL-13 by etanercept was compared to that of IL-6 and monocyte colony stimulating factor (M-CSF). The effect of etanercept on the ability of RA sera to promote DC growth was studied using DC progenitors.

Results. IL-13 was increased in RA sera versus normal sera, OA SF, and RA SF. Relative to OA SF and normal sera, RA SF was enriched in IL-13. The IL-13 contained in RA samples was biologically active, prompting DC growth from progenitors. Circulating DC growth activity was strongly reduced by anti-TNF therapy. Whereas decreases in DC growth factors including IL-13 and IL-6 occurred with etanercept therapy and were associated with clinical improvement, concurrent increases in circulating M-CSF (a non-DC, monocyte-specific growth factor) were noted.

Conclusion. The increase of biologically active IL-13 in RA supports the concept that IL-13 regulates immune cell (including dendritic cell) activity and indicates how the varied anatomical distribution of cytokines may play a role in the RA disease process. The differential regulation of circulating IL-13 and M-CSF levels by TNF antagonists further implies discrete roles in the TNF-cytokine network in RA. (J Rheumatol 2002;29:454–61)

Key Indexing Terms:
INTERLEUKIN 13
TUMOR NECROSIS FACTOR ANTAGONISTS
DENDRITIC CELLS
RHEUMATOID ARTHRITIS

Interleukin 13 (IL-13) is a pleiotropic cytokine that shares many biological activities with IL-4. IL-13 and IL-4 are positive regulators of B cell and dendritic cell (CD14 derived myeloid DC) growth and negatively regulate Th1 inflammatory responses by suppressing macrophage activation and the production and activity of inflammatory cytokines such as tumor necrosis factor (TNF)[1-5]. IL-13 and IL-4 exhibit common cell surface receptors, but IL-13-specific receptors exist (IL-13 alpha 2) that do not bind to IL-4, and that are not expressed on T cells. Thus, in contrast to IL-4, IL-13 does not appear to directly regulate the growth of Th2-type cells[1]. While IL-4 has been shown to antagonize the effects of TNF *in vitro* by inducing down-regulation and shedding of both forms of TNF receptors, IL-13 does not produce these effects[2]. In further support of non-overlapping biological activity, differences in intracellular signal transduction have been described and production of IL-13 and IL-4 by human peripheral blood cells treated with phorbol esters (phorbol myristic acetate), interferon-γ (IFN-γ), or phytohemagglutinin is regulated differently[6-8].

It has been speculated that in the inflamed rheumatoid arthritis (RA) joint, a paucity of IL-4 and the presence of IFN-γ may favor inflammatory-type Th1 responses over Th2 responses[9-11]. These observations have prompted the idea of using IL-4 as a deviator of inflammation in RA[12]. Preliminary data acquired from animal models of arthritis

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indicate that continuous administration or overproduction of IL-4 delayed the onset of clinical symptoms and histologic features of synovitis[13-15], lending credence to such strategies. Because IL-13, like IL-4, regulates Th1/Th2 responses and monocyte-macrophage secretion of inflammatory mediators, it may also modulate T cell activity and inflammation in RA[16,17]. Alternatively, IL-13 may exhibit functions that do not overlap with IL-4, as described in animal models of allergic asthma and schistosomiasis[18-20]. In contrast to IL-4, which is consistently detected at low levels in RA, there is conflicting evidence for the existence of IL-13 mRNA and protein in synovial tissue and synovial fluid[21-25]. This has limited progress in understanding the role of IL-13 in the inflamed RA joint and the potential clinical application of IL-13 as an antiinflammatory agent. Moreover, circulating IL-13 levels in RA sera, which may lend insight into systemic effects, are currently unknown.

TNF antagonist therapy in RA rapidly suppresses a variety of inflammatory mediators such as erythrocyte sedimentation rate (ESR), C-reactive protein, and proinflammatory cytokines (IL-1, TNF, IL-6)[26]. Because of the expansive cell and cytokine network prompted by TNF overproduction, in addition to inhibiting activities that are directly mediated by TNF, anti-TNF therapy might indirectly regulate other cytokines, such as IL-13. To understand the physiology of IL-13 in RA and the effects of anti-TNF therapy on IL-13, we compared levels of IL-13 in RA sera (pre/post TNF antagonist therapy) and synovial fluid (SF), osteoarthritis (OA) SF, and normal human serum (NHS). The existence of biologically active IL-13 in RA SF was substantiated by using dendritic cell (DC) progenitors that develop into mature DC in the presence of IL-13, and neutralization strategies employing polyclonal antibodies to IL-13. The modulation of IL-13 levels by TNF antagonists was compared to that of IL-6 (an inflammatory cytokine that is thought to be involved in the RA disease process and that promotes DC and monocyte growth), and monocyte colony stimulating factor (M-CSF, a monocyte growth factor known to be increased in various arthritic conditions lacking DC growth activity). Changes in the ability of RA sera to promote DC growth occurring with TNF antagonist therapy were associated with alterations in IL-13, IL-6, and M-CSF levels in the RA sera.

MATERIALS AND METHODS

Patient population and biological samples. Forty-five patients with RA, diagnosed according to the 1987 revised criteria of the American College of Rheumatology, were studied. Eleven patients were male, mean age 57 years (range 28–82). SF and peripheral blood (PB) samples were obtained as part of routine clinical care. Cell-free SF and serum were cleared of any precipitate by centrifugation (15 g for 15 min at 4°C). Ten patients with OA and 12 healthy subjects (7 women) were also included. The study was conducted according to Winthrop University Hospital institutional guidelines. Roughly 80% of the patients with RA were receiving disease modifying antirheumatic drugs. The TNF antagonist employed in this study was etanercept (Immunex/Wyeth-Ayerst, Seattle, WA, USA), a soluble TNF receptor protein that binds to TNF and neutralizes its activity. Patients were treated with subcutaneous injections (25 mg) of etanercept biweekly as recommended by the supplier. More than 80% of the patients had favorable clinical responses within one month of treatment, consistent with etanercept treatment[26,27]. This included reductions in ESR, joint swelling and tenderness, and the total number of affected joints.

Detection of cytokines in patient samples. Because reports of IL-13 levels in RA are contradictory, we independently tested RA, OA, and control samples for IL-13 by sandwich ELISA (Immunotech, Marseille, France). Proper sample storage at –80°C and handling (only one freeze-thaw cycle) were critical for accurate detection of IL-13. Although the commercially available sandwich IL-13 ELISA we employed contains diluent designed to minimize possible interference caused by rheumatoid factor (RF), we conducted additional studies designed to test the potential effect of RF present in RA sera and SF on IL-13 determination. We compared IL-13 levels in RA samples with no to very high RF titers, performed IL-13 recovery tests after spiking samples with 2 different concentrations of IL-13, and preincubated RA samples with isotype matched nonimmune antibody (to block binding of RF to Ig Fc regions). Measurement of IL-13 was not influenced by RF. Preincubation of RA samples with isotype matched antibodies did not alter IL-13 detection and the recovery of known amounts of IL-13 was not affected by the presence of RF in RA samples. Of note, there was no interference with the detection of spiked IL-13 in RA SF, which often contains higher RF levels than paired RA sera[28-30]. The detection of high IL-13 levels (240 and 500 pg/ml) in RA patient sera that were RF negative also argues against RF interfering with accurate IL-13 measurement in this assay. IL-6 and M-CSF levels were also detected by ELISA (R&D Systems Inc., Minneapolis, MN, USA) and were not influenced by RF, determined as described above for IL-13. All ELISA assays were performed exactly as recommended by the manufacturer. The sensitivity for IL-13 was < 1.5 pg/ml, for M-CSF < 9.0 pg/ml, and for IL-6 < 0.70 pg/ml.

IL-13 bioactivity assay. The existence of biologically active IL-13 in RA SF was examined using hematopoietic progenitors (myelodendritic cells) that develop into DC in the presence of IL-13[31,32] and with neutralizing polyclonal antibodies to IL-13 (Becton-Dickinson/PharMingen, San Diego, CA, USA). Myelodendritic cells are intermediate (CD34–CD33+ DR+CD115+) progenitors of the CD14 derived DC pathway in the cord blood model[31] and differentiate into either monocytes, granulocytes, or DC when cultured with M-CSF, granulocyte-CSF (G-CSF) or CD14-DC growth factors [GM-CSF/IL-4 or GM-CSF/IL-13 +/– stem cell factor (SCF) +/– TNF], respectively. They were obtained by treating GM-CSF/TNF/SCF instituted cord blood cultures on Day 3 with 15 μg/ml of rabbit polyclonal anti-TNF antibody (Genzyme, Cambridge, MA, USA), as described[31,32]. Following anti-TNF antibody treatment, cultures were supplemented with fresh NHS/RPMI media without exogenous cytokines on a weekly basis to maintain myelodendritic cells in a progenitor state. After 10–21 days, myelodendritic cells were removed from culture, centrifuged, and adjusted to 0.4×10^5 cells/ml in fresh serum-free RPMI 1640 medium containing 2 mM L-glutamine, 10 mM HEPES, 50 IU/ml penicillin, 50 μg/ml streptomycin, and RA sera, untreated RA SF, or RA SF pretreated with anti-IL-13 antibody (200 ng/ml, Pharmingen) for 45 min at room temperature. In control cultures, nonimmune antibody was substituted for anti-IL-13. Development of mature DC was assessed by morphology using phase microscopy, by expression of CD86, CD1a, and class II MHC antigens, and by activation of naïve T cells, as described below.

Dendritic cell functional assays. The ability of mature DC obtained from myelodendritic cells in the presence of RA sera (pre/post etanercept therapy) and RA SF +/– anti-IL-13 to stimulate T cell proliferation was tested in an allogeneic mixed leukocyte reaction (MLR) using nylon wool nonadherent T cells obtained from healthy volunteers as responders, as described[31,32]. T cell proliferation was measured on Days 6–7 by adding 0.5 μCi of [^{3}H] thymidine to cells and harvesting the cells after 5 h using an

automated sample harvester. Counts per minute were measured in a liquid scintillation counter. Results are expressed as the mean of triplicate samples ± standard error.

Detection of intracellular T cell cytokines associated with Th1/Th2/Th0 responses was as described[32]. Briefly, on Day 6–7, cells present in the MLR cultures were resuspended in fresh 5% NHS/RPMI media at 2×10^6 cells/ml. For T cell restimulation and the intracellular retention of cytokines, cells were incubated in 25 ng/ml PMA (Sigma), 1 μg/ml ionomycin (Sigma), and 5 μg/ml Brefeldin A (Sigma) for 4 h in a 5% CO_2 incubator. Cells were then incubated with anti-CD3 PerCP (Becton Dickinson), permeabilized (FACS lysing and permeabilizing solutions, Becton Dickinson), and stained with FITC-anti-IFN-γ (Th1-restricted) and PE-anti-IL-4 (Th2-restricted) antibodies (Becton Dickinson). Isotype control samples received mouse IgG1 labeled with phycoerythrin (PE) and FITC instead of anti-cytokine antibodies. After staining, cells were fixed in 10% buffered formalin in phosphate buffered saline and stored in the dark until analyzed. Roughly 15–30,000 cells were collected on the FACScan as indicated above. Calbrite beads (Becton Dickinson) were used to calibrate the FACScan for FITC, PE, and PerCP. Region analysis was set according to CD3 reactivity, positive values were determined according to isotype controls.

Statistical analysis. The Student t test or Mann-Whitney rank sum test was used to analyze data using Sigma Stat software (Jandel Scientific, San Rafael, CA, USA).

RESULTS

Distribution of IL-13 in RA SF, RA sera, normal human sera (NHS), and OA SF. IL-13 levels in RA sera were significantly increased versus NHS (758 vs 4.6 pg/ml; $p < 0.0001$) (Figure 1A). None of the 33 RA sera tested fell below the lower limits of detection, whereas 9/12 NHS samples did. IL-13 levels in OA SF (n = 10) were not increased versus NHS, except in one patient (data not shown). Based on specific clinical findings, this OA patient was being evaluated for a comorbid inflammatory condition. Although IL-13 levels were significantly lower in RA SF than in RA sera (103 vs 758 pg/ml; $p = 0.006$), the amount of IL-13 was increased in RA SF versus NHS ($p < 0.004$) and OA SF ($p = 0.004$). In contrast to IL-13, which was detected at higher levels in RA sera than in RA SF, higher levels of IL-6 were noted in RA SF than in RA sera (Figure 1B) (275 vs 42 pg/ml; $p = 0.003$). These observations are consistent with the proposed role of IL-6 in the RA inflammatory process[33].

IL-13 present in RA SF is biologically active. Previously, we demonstrated that cell culture media containing RA SF and RA sera, and lacking exogenous cytokines, sustains the *in vitro* maturation of DC from myelodendritic progenitors exhibiting DC developmental potential. Cell intermediates present in these cultures coexpressing CD14 and CD1a denote specific components of the CD14 derived DC pathway[32]. Mature DC arising from myelodendritic progenitors express high levels of class II MHC (DR) and CD86, and are thus potent stimulators of an allogeneic MLR[32]. Because IL-13 (like IL-4) is a preferred myeloid DC growth factor, we speculated that IL-13 in RA SF and serum might contribute to the DC growth effects we have reported[32]. To determine if the IL-13 detected by ELISA in RA samples is biologically active, we compared the ability of untreated RA

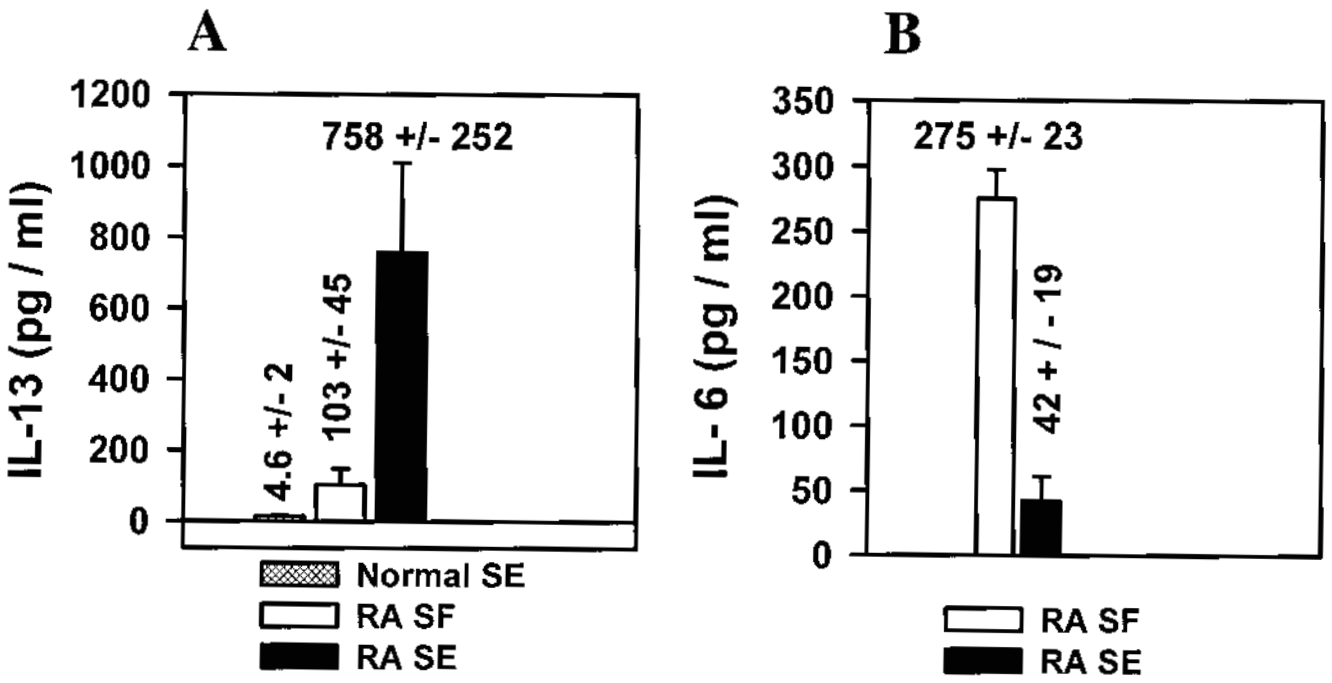

Figure 1. A. The distribution of IL-13 levels in RA synovial fluid (RA SF, n = 13), RA sera (RA SE, n = 33), normal human sera (normal SE, n = 12). IL-13 in RA sera was increased by > 100-fold versus normal SE. While IL-13 levels in RA sera were higher than in RA SF, RA SF contained higher levels of IL-13 than OA SF. B. IL-6 levels in RA SF and RA SE (each n = 10). In contrast to IL-13, which was detected at higher levels in RA sera than in RA SF, higher levels of IL-6 were noted in RA SF than in RA sera. Because IL-6 is an important mediator of inflammation and an accessory cytokine for dendritic cell growth, the higher levels of IL-6 in RA SF are consistent with a local inflammatory process.

SF and RA SF pretreated with neutralizing polyclonal antibodies to IL-13 to induce DC growth from myelodendritic progenitor cells. As expected, untreated RA SF promoted the growth of mature DC that were strong stimulators of naïve T cells in the MLR (Figure 2). Neutralization of IL-13 activity in RA SF with anti-IL-13 resulted in a diminished ability of RA SF to yield mature DC from myelodendritic progenitor cells, as shown by reduced T cell proliferation in the mixed leukocyte (~3-fold, n = 3) (Figure 2). Specific interference with DC growth was further evidenced by the lack (≤ 1.5%) of CD86+DR+ and CD14+CD1a+ cells and the scarcity of cells exhibiting DC morphology in cultures containing RA SF pretreated with anti-IL-13 antibody. Control nonimmune antibody did not produce these effects.

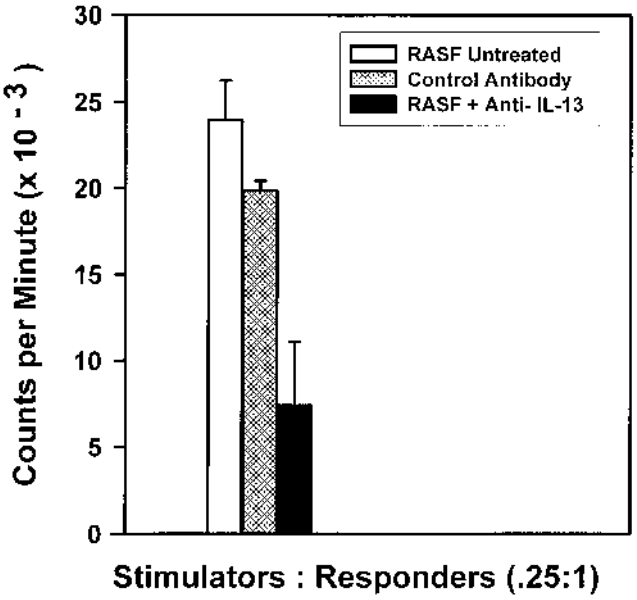

Figure 2. IL-13 present in RA synovial fluid (SF) is biologically active. IL-13 bioactivity was tested on dendritic cell growth in neutralization assays using dendritic cell progenitors (myelodendritic cells). Myelodendritic cells were cultured with untreated RA SF or RA SF pretreated with anti-IL-13 antibody for 45 min. After 5 days the ability of the cultured cells to stimulate a mixed leukocyte reaction was tested. The mean ± SE of 3 experiments testing different SF is shown. Isotype control Ig (rat IgG1) used at the same concentration was not inhibitory ($p > 0.05$ vs anti-IL-13 antibody).

TNF antagonist therapy selectively alters circulating levels of IL-13, IL-6, and M-CSF. Analysis of 20 paired RA sera pre- and post TNF antagonist (etanercept) therapy revealed significant reductions in IL-13 levels after 2–4 weeks (237 vs 112 pg/ml; $p = 0.02$) (Figure 3A). Continued drops in IL-13 occurred after 4 months (data not shown). Despite the large discrepancy between IL-6 levels in RA SF and RA sera and the relatively low levels of IL-6 in RA sera, TNF antagonist therapy still produced significant decreases in circulating IL-6 levels after 2–4 weeks (42 vs 4.6 pg/ml; $p = 0.002$) (Figure 3B). Consistent with these results, another class of TNF antagonist, an anti-TNF-specific antibody (infliximab), produces reductions in serum IL-6 levels shortly after the onset of therapy[33]. We did not observe differences in the levels of IL-1α or IL-1ß pre- and post therapy (data not shown). In contrast to the reduction of circulating IL-13 and IL-6, etanercept produced increases in circulating M-CSF (Figure 3C). M-CSF levels were increased (1621 vs 745 pg/ml; $p = 0.047$) in 8 of 11 paired RA sera samples tested (in the remaining 3 paired samples no changes occurred). These increases represent greater than twice the levels reported for normal human sera (670 pg/ml; R&D Systems).

Decreases in IL-13 and IL-6 levels in RA sera with etanercept therapy are linked with decreased potential of the sera to promote DC growth. Comparisons of phase contrast microscopic examinations of myelodendritic progenitor cell

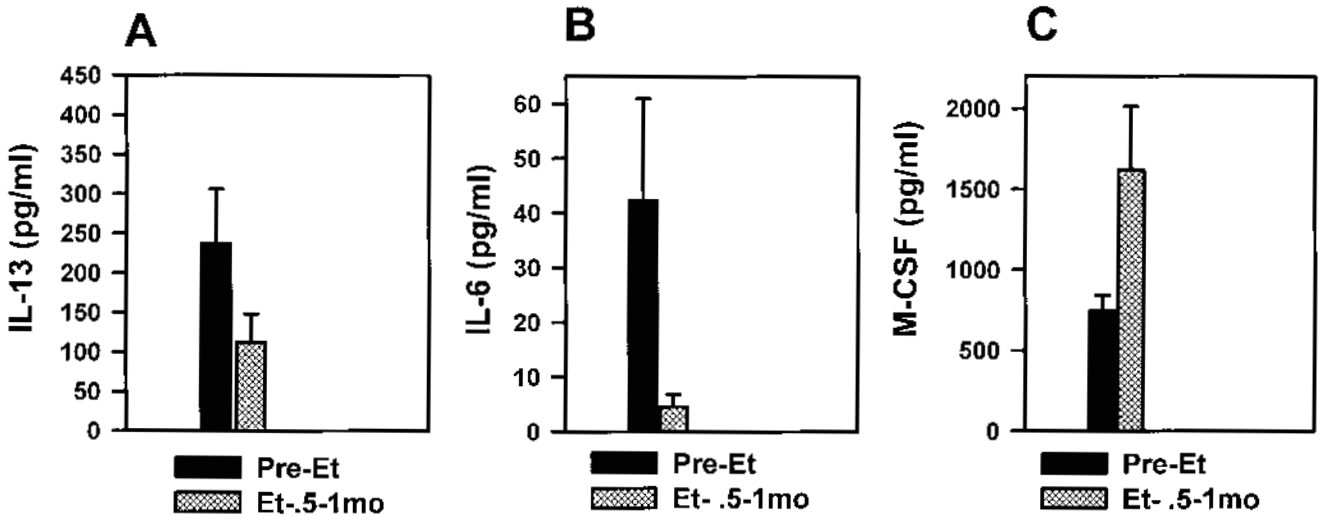

Figure 3. Differential regulation of circulating IL-13, IL-6, and M-CSF by etanercept (Et). Analysis of paired RA sera before and 0.5–1.0 month after etanercept revealed reductions in IL-13 (A, n = 20) and IL-6 (B, n = 11) and concomitant increases in M-CSF (C, n = 8) after therapy.

cultures containing RA sera before and after etanercept therapy revealed typical DC with pre-etanercept sera, but not with post-etanercept sera (Figure 4). Functional analysis of cells obtained from these cultures in MLR and Th1/Th2/Th0 assays further substantiated decreased circulating DC growth activity with TNF antagonist therapy. Sera from 5 of 6 patients with RA tested pre- and post therapy (2–4 weeks) exhibited a decreased potential to sustain the growth of mature DC capable of producing strong MLR (Figure 5A). Analysis of the class of T cell response generated in the MLR by intracellular staining revealed a predominance of inflammatory Th1 cytokines (IFN-γ) over Th2 (IL-4) cytokines before etanercept therapy, as we reported[32]. After 2–4 weeks of etanercept therapy, the ability of RA sera to yield DC progeny exhibiting the capacity to promote Th1-type responses was greatly reduced (Figure 5B). Treatment of myelodendritic cells with GM-CSF + IL-4 or IL-13 yielded myeloid DC that produced strong MLR and Th1 responses, and treatment with NHS sustained a progenitor state, as expected (data not shown)[31]. Because IL-13 and IL-6 are DC growth factors[1,3,4,34], these results support the idea that TNF antagonist therapy modulates DC activity in RA.

DISCUSSION

To our knowledge, this is the first report to describe that (1) IL-13 levels are significantly increased in the sera of patients with RA compared to normal sera, OA SF, and RA SF; and (2) TNF antagonist therapy modulates DC growth activity in RA. RA SF was significantly enriched in IL-13 relative to OA SF. IL-13 present in the RA samples was biologically active, as determined by neutralization of DC growth activity with anti-IL-13-specific antibodies. Therapy of RA patients with TNF antagonist (etanercept) produced distinct alterations in circulating cytokine levels that were associated with clinical improvement. Whereas decreases in circulating IL-13 and IL-6 occurred with etanercept, concurrent increases in M-CSF levels were noted. These effects were further associated with a loss of circulating DC growth

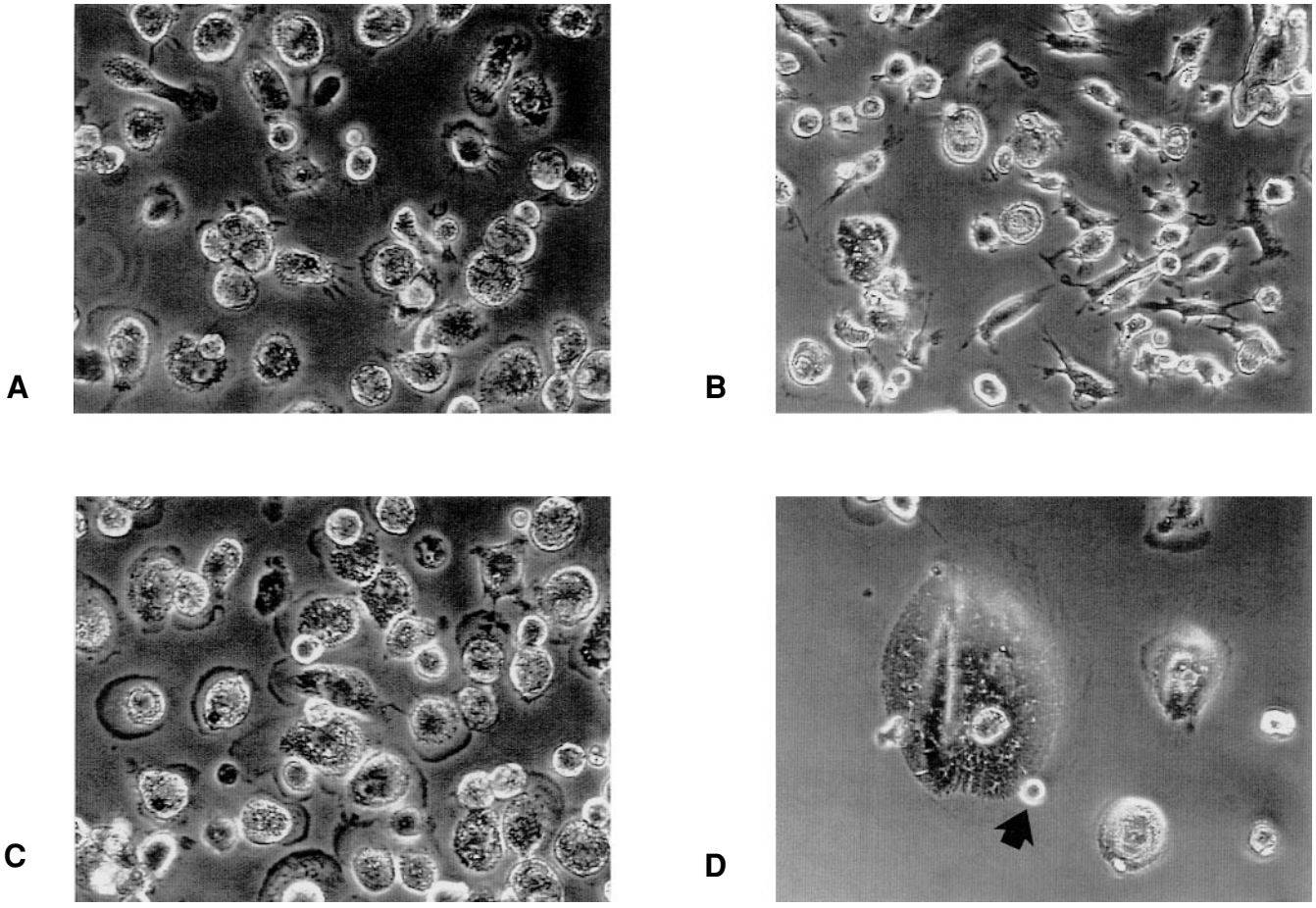

Figure 4. RA sera obtained after 2–4 weeks of therapy are incapable of yielding progeny exhibiting typical dendritic cell (DC) morphology. Treatment of myelodendritic progenitors with pre-etanercept sera produced cells with typical DC features, as shown in cultures on Days 3 (A) and 10 (B). RA sera obtained after 2 weeks of etanercept therapy (C, D) produced cells that resembled large monocyte-macrophages (many with foamy cytoplasm) and few DC (C = Day 3 cultures, D = Day 10 cultures), which is consistent with the observed increased M-CSF and decreased IL-13 in the sera. Arrow, D, points to an undifferentiated myelodendritic progenitor cell. One of 6 typical comparisons at 2–4 weeks is shown. Original magnification ×40.

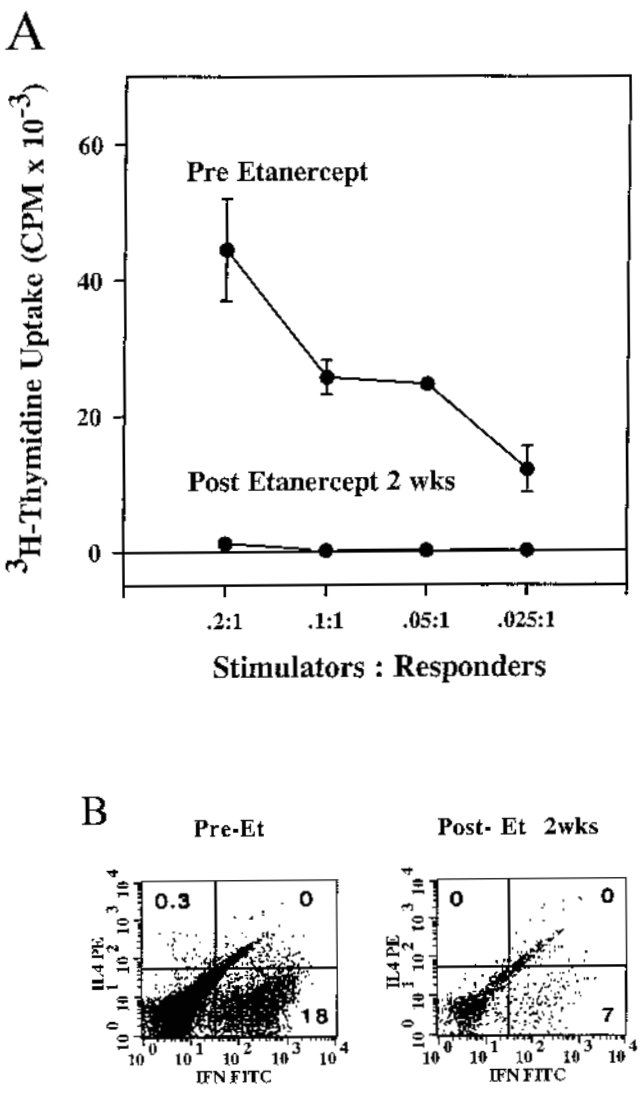

Figure 5. RA sera after etanercept therapy reveal a reduced capacity to yield dendritic cells (DC) that stimulate mixed leukocyte reactions and Th1-type responses. Myelodendritic progenitor cells were cultured for 5 days in pre- or post-etanercept sera; cells were then tested as stimulators of mixed leukocyte reactions (A) and Th1 (IFN-γ)/Th2 (IL-4) responses (B). Duplicate determinations of the same patient samples tested on different days yielded similar results. Reductions in DC growth activity occurred with 5/6 sera studied. Results shown represent a typical experiment from one of those 5.

activity.

The lack of IL-13 receptors on T lymphocytes[1] implies that the increased IL-13 in RA sera does not directly promote Th2 growth. Instead, the high levels of circulating IL-13 in patients with RA may indirectly inhibit the development of Th1 responses in the periphery by suppressing the production of inflammatory cytokines from monocytes. Other IL-13 driven nonlymphocyte functions, such as the recruitment of immature DC and monocytes into the periphery, may also occur via specific chemokine /chemokine receptor systems (IL-8 and their ligands)[35]. Recently, Bonecchi, *et al* showed that IL-13 upregulates the expression of functional IL-8 receptors (CXCR1 and CXCR2) on monocytes and DC[35]. Instead of preferentially promoting neutrophil chemotaxis into areas of chronic inflammation such as the RA joint, these authors proposed that the upregulation of IL-8 by IL-13 selectively prompts the migration of monocytes into sites of inflammatory lesions. In support of these speculations, we and others have demonstrated high levels of chemokine receptors, including CXCR1, on RA peripheral blood monocytes and DC precursors[36].

We detected higher levels of soluble IL-13 in RA SF compared to OA SF, which is in agreement with Isomaki, *et al*[21]. However, the levels of IL-13 we observed were far lower (our mean 103 ± 45 vs 1307 ± 247 pg/ml) than reported by those investigators. Our findings are not necessarily in disagreement with those of Kotake, *et al*[22] and Woods, *et al*[23], who independently report a lack of IL-13 mRNA in the RA joint. They instead point to the possibility that IL-13 protein may not be consistently or uniformly produced in the RA joint, but may be derived mostly from the circulation. If IL-13 enters the joint via the circulation, it would not be inconsistent to find lower levels of IL-13 mRNA and/or varying levels of IL-13 protein within the joint environment.

We have reported that RA sera and RA SF contain myeloid DC growth factors and proposed that IL-13 contributed to these effects[32]. Our current findings demonstrating the presence of biologically active IL-13 in RA SF that sustains myeloid DC growth (Figure 2) support this concept. Conceivably, biological activity of IL-13 within the joint may involve synergy between IL-13 and other myeloid DC growth factors (such as TNF, GM-CSF, and stem cell factor). Because RA joint associated myeloid DC have been shown to selectively promote Th1 responses associated with chronic inflammation[32], our results indicate that the presence of IL-13 in the RA joint contributes to local immunopathology, at least in part by promoting DC development. B cells may also be targets of IL-13 activity in specific joint microenvironments[1].

While TNF antagonists are known to suppress the production of a variety of mediators associated with chronic inflammation[26], the effect of TNF antagonist therapy on noninflammatory cytokines such as IL-13 and on DC growth regulation had not been investigated. We associated decreased IL-13 and IL-6 levels with a lack of DC growth activity in post-etanercept sera and noted increases in M-CSF in the same sera (Figures 3–5) in patients responding favorably to etanercept. M-CSF is a cytokine that promotes the growth of monocytes lacking antigen-presenting cell functions and that does not sustain DC growth[31,32]. While high levels of M-CSF have been reported in the RA joint, other arthritic conditions such as OA also exhibit elevated levels of M-CSF, suggesting that M-CSF does not play a

unique role in RA pathogenesis[37]. We propose that with decreasing levels of IL-6 and IL-13 (and other proinflammatory/DC cytokines such as TNF and GM-CSF) a rise in M-CSF may be a biological response that disrupts DC growth. The effects of TNF antagonists on DC development we report are consistent with a key role of TNF in DC hematopoiesis[38]. Since myeloid DC preferentially activate Th1 inflammatory responses and RA is associated with enhanced Th1 responses, the early efficacy of etanercept therapy may be in part directly related to its ability to limit the yield of myeloid DC.

Our results substantiate that IL-13 levels are increased in RA, especially in the circulation, and provide new insight into how differences in the anatomical distribution of cytokines may play a role in the RA disease process. In the joint, the relatively lower levels of IL-13 may be conducive to the development of Th1 responses. In the circulation, the principal activity of IL-13 may be to modulate the recruitment and growth of monocytes and immature DC, rather than to inhibit Th1 responses. Because IL-4 has been consistently shown to be present at low levels in RA, IL-13 and not IL-4 may be the principal mediator of IL-4/IL-13 driven immune activity RA. The varied effect of TNF antagonists on circulating IL-13 and M-CSF levels further indicates that noninflammatory cytokines participate in the TNF-cytokine network in RA.

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