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J Clin Invest. 1991;87(3):1108-1113. https://doi.org/10.1172/JCI115073.

Research Article

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Transforming Growth Factor β1 Suppresses Acute and Chronic Arthritis in Experimental Animals

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Abstract

Systemic administration of the cytokine, TGFβ1, profoundly antagonized the development of polyarthritis in susceptible rats. TGFβ1 administration (1 or 5 μg/animal), initiated one day before an arthritogenic dose of streptococcal cell wall (SCW) fragments, virtually eliminated the joint swelling and distortion typically observed during both the acute phase (articular index, AI = 2.5 vs. 11; $P < 0.025$) and the chronic phase (AI = 0 vs. 12.5) of the disease. Moreover, TGFβ1 suppressed the evolution of arthritis even when administration was begun after the acute phase of the disease. Histopathological examination of the joint revealed the systemic TGFβ1 treatment greatly reduced inflammatory cell infiltration, pannus formation, and joint erosion. Consistent with the inhibition of inflammatory cell recruitment into the synovium, TGFβ1 reversed the leukocytosis associated with the chronic phase of the arthritis. Control animals subjected to the same TGFβ1 dosing regimen displayed no discernable immunosuppressive or toxic effects even after 4 wk of treatment. These observations not only provide insight into the immunoregulatory effects of TGFβ, but also implicate this cytokine as a potentially important therapeutic agent. (*J. Clin. Invest.* 1991. 87:1108–1113.) Key words: cytokine • inflammation • immunosuppression • leukocytosis • leukocyte

Introduction

The potent immunosuppressive effects of the cytokine, TGFβ, suggest that it may be valuable in the treatment of disease states characterized by aberrant function of the immune system. TGFβ has been shown in vitro to inhibit proliferation of thymocytes (1), T and B lymphocytes (2–5), and the less differentiated hematopoietic progenitor cells (6–9). In addition, it suppresses B cell production of IgG and IgM (2) and antagonizes the immunoregulatory effects of interleukins-1, -2, and -3 (2–4, 10), colony stimulating factors (8, 10), and interferons alpha (11) and gamma (12). Although TGFβ has been shown in vitro to have a myriad of immunosuppressive effects, its ability to block immune responses in vivo, in particular, chronic inflammatory lesions, has not been evaluated. Since many of the immune cell functions influenced by TGFβ are involved in the sequence of events leading to connective tissue destruction in arthritic lesions, the ability of TGFβ to inhibit these pathways may be effective in suppressing the pathogenesis of this chronic inflammatory disease.

This study examines the effectiveness of systemic administration of TGFβ1 in altering the progression of an inducible arthritis in experimental animals. A single injection of streptococcal cell wall (SCW)[1] fragments into susceptible rats induces an acute inflammation of the joints, followed by the development of chronic proliferative and erosive disease (13, 14). The chronic arthritic condition has been identified as a T cell and monocyte-mediated immune response (15) and thus could potentially be modulated by an immunosuppressive agent such as TGFβ. The results of this study show that daily administration of TGFβ1 sharply curtails the evolution of both the acute and chronic phases of the disease. Furthermore, the same TGFβ1 dosing regimen did not have noticeable toxic or suppressive effects on normal animals.

Methods

Reagents. TGFβ1 was purified to homogeneity from bovine bone (16, 17) and by SDS-PAGE migrated as a single band. TGFβ1 was dissolved in 12 mM HCl, 20% ethanol (2.5 μg TGFβ1/μl, stock) and stored at −70°C in aliquots. Rat serum albumin (RSA; Sigma Chemical Co., St. Louis, MO) was dissolved in 8 M urea, 10% acetic acid, pH 3.5 (10 mg/ml), and filtered (0.45 μm). The albumin was eluted from a C18 reverse-phase HPLC column (2.2 × 25 cm; Vydac, Hesperia, CA) with a linear acetonitrile gradient (22.5 to 54% [vol/vol]) in 0.1% (vol/vol) trifluoroacetic acid, a procedure that previous studies had shown to cause a 99% reduction in the endotoxin levels as determined by Limulus amebocyte lysate assay (18). The RSA was lyophilized and stored at 4°C.

Arthritis induction and TGFβ1 administration. Specific pathogen-free Lewis female rats (~ 100 g) (Harlan Sprague Dawley, Inc., Indianapolis, IN) were injected with peptidoglycan-polysaccharide fragments (30 μg rhamnose/g body mass) derived from group A SCW to induce an erosive polyarthritis as previously described (13, 14). The arthritic response was quantified by determining the articular index (AI). Each of the four distal joints was scored blinded on a scale of 0–4 on the basis of swelling, redness, and degree of deformity of normal contours. The individual scores were summed to arrive at the whole animal score, a possible maximum of 16. Joint scores (AI) were averaged for each

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Received for publication 27 September 1990 and in revised form 27 November 1990.

1. *Abbreviations used in this paper:* AI, articular index; RSA, rat serum albumin; SCW, streptococcal cell wall.

group of animals and reported as average±SEM, unless otherwise indicated. Statistical significance was ascertained using the Student's *t* test. In individual experiments, groups consisted of two to five animals.

TGFβ1 was intraperitoneally injected daily for intervals specified for each experiment, up to 32 d. The TGFβ1 stock was diluted in a vehicle of RSA in PBS (1 mg/ml) to 0.1–5.0 μg TGFβ1/2 ml vehicle immediately before intraperitoneal administration. Control animals received an equal volume (2 ml) of either the vehicle or PBS. The vehicle was found to contain ≤ 25 pg/ml endotoxin (limit of detection) (18).

Light microscopy. At defined intervals during the evolution of the arthritic response, joint tissues from control and arthritic rats were excised and fixed in a solution of 10% buffered formalin, embedded in paraffin, sectioned (8 μm), and stained with hematoxylin and eosin. Tissue to be used for antibody staining to identify SCW deposits was fixed and sectioned in a similar manner, then treated as before (19).

Cell isolation, culture, and analysis. At selected intervals, blood smears, hematocrits, and total white cell counts (ZBI Coulter Counter; Coulter Electronics, Inc., Hialeah, FL) were obtained for each animal. At the time of tissue harvest, PBMCs were isolated from heparinized blood by density gradient centrifugation through Histopaque 1083 (Sigma Chemical Co.). Spleen cells were obtained as described (5), suspended in DME (Mediatech, Inc., Washington, DC) containing 50 μg/ml gentamicin sulfate, 2 mM glutamine, 0.5 μM β-mercaptoethanol, and 1% rat serum, and plated in 96-well flat-bottomed plates (Costar Corp., Cambridge, MA) (4×10^5/200 μl per well). Proliferation was assessed in the presence or absence of stimuli: ConA (Calbiochem-Behring Corp., San Diego, CA) and PHA (Burroughs Wellcome Co., Greenville, NC), as previously described (5, 15). After 68 h of culture, the cells were pulsed for 4 h with 0.5 μCi/well of [^{3}H]thymidine ([^{3}H]TdR, sp act 6.7 Ci/mmol) (Schwarz/Mann, Orangeburg, NY). The cultures were harvested using an automated harvester (Skatron, Inc., Sterling, VA) and the amount of incorporated radioactivity was determined in a liquid scintillation counter (1205 Beta-plate; Pharmacia, Inc., Gaithersburg, MD).

Results

Suppression of acute and chronic arthritis by TGFβ1. To assess the effects of TGFβ as a therapeutic agent for arthritis, TGFβ1 was administered daily to Lewis rats at doses of 0.1, 1.0, and 5.0 μg/100 g rat, beginning 1 d before the injection of SCW which initiated the arthritis. SCW-treated animals, which did not receive TGFβ1, displayed the acute and chronic joint swelling and deformity which is typical of SCW-induced arthritis (Fig. 1). However, when 5 μg of TGFβ1/rat was administered daily during the evolution of the arthritic response, the animals displayed a very blunted acute inflammatory phase (AI = 2.5±1.5 vs. 11±0.9; $P < 0.025$; day 5) and virtually no chronic inflammatory phase (AI = 0 vs. 12.5±0.5; day 21). The striking diminution of the acute and chronic components of the evolving SCW-induced polyarthritis was also noted in those animals that received only 1 μg TGFβ1 daily. These animals displayed minimal joint inflammation during the acute phase of the arthritis (AI = 1.8±1.2; $P < 0.005$) and during the chronic phase as well (AI = 1.8±1.0; $P < 0.005$). Even a dose as low as 0.1 μg TGFβ1/ animal daily resulted in a decrease in the severity of the joint inflammation during the acute and chronic phases and a delay in the onset of the chronic inflammation (Fig. 1). Control animals not receiving SCW, but dosed intraperitoneally daily with TGFβ1 (5 μg/animal), vehicle (1 mg RSA/ml PBS), or PBS had no synovial pathology.

Suppression of established arthritis by TGFβ1. Because of the profound effect of TGFβ1 on the evolution of arthritic lesions when administration was begun before the onset of detectable inflammation, we next evaluated whether TGFβ1 could independently suppress chronic inflammatory events. TGFβ1 administration (5 μg/animal per day) was begun on

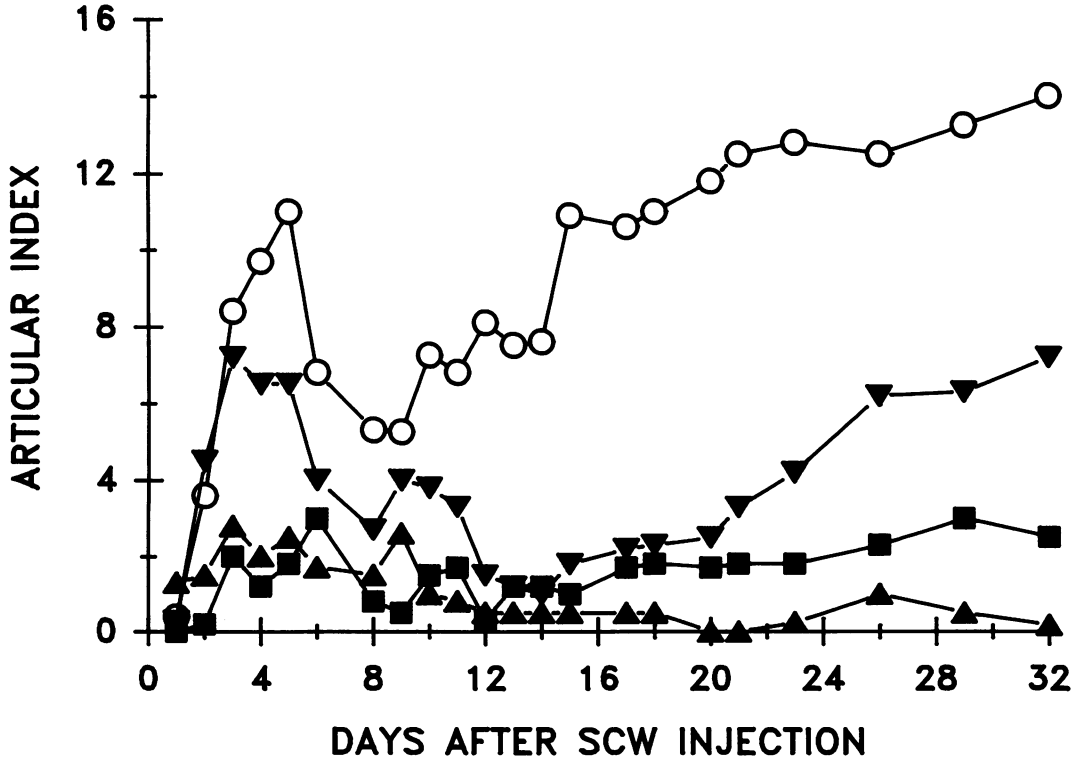

Figure 1. Modulation of SCW-induced arthritis by various doses of TGFβ1. Animals were injected with SCW on day 0. Some animals received no additional treatment (*circles*); others were treated with TGFβ1 (i.p.) daily at 0.1 (*inverted triangles*), 1.0 (*squares*), or 5.0 μg/animal (*triangles*) beginning the day before SCW injection. Each point represents the mean joint score for each group of animals (n = 3–5). Data for all groups of control animals (PBS, vehicle, and TGFβ1-injected) are not shown; all control animals had mean joint scores of zero throughout the experiment. The experiment with some modifications was repeated three times with similar results.

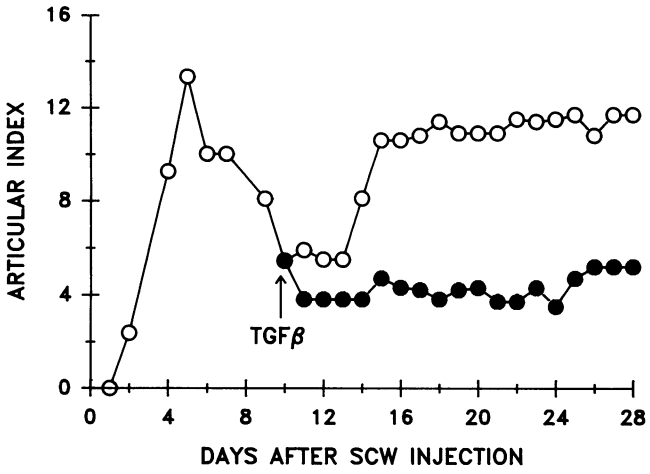

Figure 2. The effect of TGFβ1 treatment on the chronic phase of the arthritis. Lewis rats were injected on day 0 with SCW and their AI determined daily thereafter. On day 10, half of the animals (*filled circles*) were begun on a daily dosing regimen of 5 μg TGFβ1/animal (i.p.). Joint scores for control animals were as indicated in Fig. 1.

day 10 for a group of SCW-injected animals and a group of control animals and continued for 18 d (Fig. 2). Before this point, all animals had very similar AI scores (AI = 5.4±0.7; day 10). However, once daily administration of TGFβ1 was begun, the scores of the treated group diverged from that of the untreated group. TGFβ1 effectively suppressed the chronic phase of the arthritis. On day 21, the AI of the untreated group was 10.9±0.9 while that of the TGFβ1 treated group was 3.7±0.7; $P < 0.0001$. Additional studies examined the timing with which TGFβ1 must be administered to avert the acute and chronic inflammation. One study examined the effect of TGFβ1 on arthritic lesions after connective tissue destruction was already apparent. When daily injections of TGFβ1 were begun on day 21, well into the chronic destructive phase, no significant change occurred in the AI of the animals (AI = 13.3±0.3 for SCW animals vs. AI = 11.0±2.0 for SCW + TGFβ animals; day 30). Furthermore, a single injection of TGFβ1 1 d before SCW administration did not diminish the acute or chronic phases of the arthritis (AI = 10.4±0.8 for SCW animals vs. AI = 10.2±0.3 for SCW + TGFβ animals; day 21).

Histopathological analysis. In order to define the potential mechanisms whereby TGFβ1 reversed the inflammatory processes leading to joint destruction, cellular events were monitored by histopathologic evaluation. The joint tissues from SCW-treated animals exhibited the lesions that typify chronic erosive arthritis: extensive synovial hyperplasia with an accumulation of mononuclear cells (macrophages, lymphocytes, and fibroblast-like cells), pannus invasion into the bone with subsequent bone erosion and cartilage destruction, and fibrous tissue replacement of the joint space (Fig. 3 *B*). However, treatment of SCW-injected animals with 5 μg of TGFβ1 eliminated the inflammation and joint erosion and the synovial tissue exhibited relatively normal morphology (Fig. 3 *C*). The synovial tissue from animals treated with 1.0 and 0.1 μg of TGFβ1 was slightly hypertrophied with an accumulation of mononuclear cells (not shown); however, the cellular infiltrate was very limited relative to that of the untreated SCW animals. In addition,

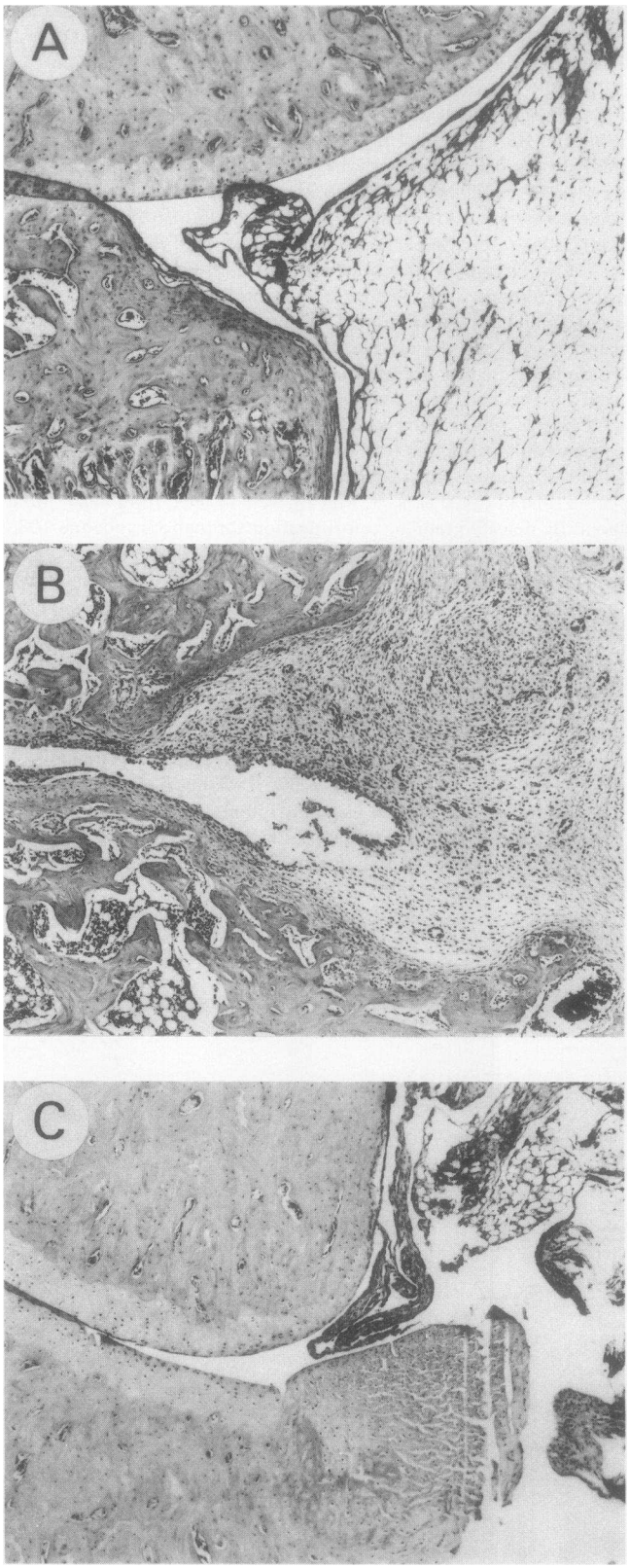

Figure 3. Histologic comparison of joint and synovial tissue after SCW and TGFβ1 treatments. Joint tissue from control rats (*A*) and rats injected with SCW (*B* and *C*) was harvested 33 d after SCW injection. Some of the animals were dosed daily with 5 μg TGFβ1 (*A* and *C*). Magnification, 16.

there was no apparent bone or cartilage erosion. Tissue from control animals injected daily with vehicle or TGFβ1 (5 μg) (Fig. 3 *A*) exhibited normal morphology. TGFβ1 administration did not appear to affect dissemination or deposition of the injected SCW as shown by immunoperoxidase staining of tissue with an antibody to SCW, nor did it appear to change the retention time of the SCW in the tissues (not shown).

Inhibition of leukocytosis by TGFβ1. The marked reduction in inflammatory cell infiltrate prompted subsequent analysis of the effects of TGFβ1 on circulating hematopoietic cells. On day 4, the number of circulating WBCs was elevated for all SCW-treated animals, regardless of any TGFβ1 treatment: $21.9\pm1.5 \times 10^3/mm^3$ ($n = 20$) for all SCW-injected animals vs. $7.0\pm0.3 \times 10^3/mm^3$ ($n = 14$) ($P < 0.0001$) for controls (Fig. 4 *A*). However, by day 32, daily systemic administration of TGFβ1 had significantly suppressed the elevated WBC count associated with the inflammation in a dose-dependent manner (Fig. 4 *B*). Hematocrits measured on day 4 and 32 were not suppressed (data not shown).

Absence of side effects in TGFβ1-treated animals. In spite of the impact of systemically administered TGFβ1 on arthritis, it had no apparent side effects nor did it cause generalized immune suppression. Spleen cells isolated from TGFβ-treated control animals incorporated equivalent amounts of [^{3}H]TdR (118,700±1,600 cpm) when stimulated with ConA as the vehicle-treated control animals (125,800±16,000 cpm). All SCW-treated animals, treated and not treated with TGFβ, exhibited suppressed spleen cell proliferation, as reported (5). PBMC isolated from the various groups of animals followed the same trends. TGFβ also did not affect the normal weight gain of the control rats (TGFβ-treated 164.0±2.3 g vs. vehicle-treated 165±3.1 g; day 32), nor did it alter the slower rate of weight gain typically observed in SCW-treated animals: 142.4±4.0 g ($n = 5$) for SCW rats compared with 149.2±6.1 g ($n = 5$) for SCW rats treated with TGFβ1. TGFβ1 injections to control animals did not cause noticeable gross or microscopic pathologies, however daily intraperitoneal injections of TGFβ1 to the SCW-injected animals exaggerated the production of connective tissue in the abdominal wall at the injection sites and surrounding the organs of the abdominal cavity.

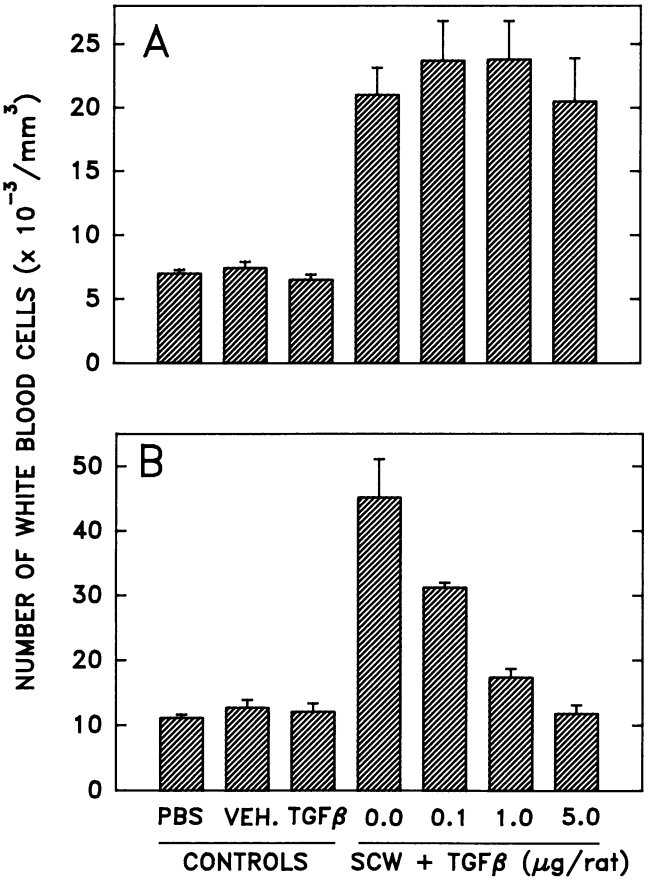

Figure 4. Alterations in number of circulating WBCs after TGFβ1 treatment. Total WBC count for control and SCW-injected animals were determined on day 4 (*A*) and day 32 (*B*). Some of the animals were treated daily with TGFβ1 daily as described in Fig. 1. The data is expressed as mean number of WBCs $\times 10^{-3}/mm^3$ blood±SEM (n = 3–10) for each group.

Discussion

Daily intraperitoneal administration of TGFβ1 to SCW-injected animals resulted in a marked suppression of the acute and chronic phases of SCW-induced arthritis. An intraperitoneal route of cytokine delivery was chosen over intravenous injection because the serum component, α_2-macroglobulin, is known to effectively bind TGFβ (20). In addition, first-pass hepatic extraction has been demonstrated to efficiently eliminate the molecule from the blood (21). By intraperitoneal administration, the retention time of the bioactivity was clearly sufficient to dramatically influence the course of events responsible for joint destruction.

The decreased inflammatory cell recruitment into the synovium of the TGFβ1-treated animals may be due to an inhibition of the SCW-induced leukocytosis. SCW-treated animals typically manifest an increased number of circulating leukocytes which serve as a reservoir of cells for recruitment into the joints and other sites of chronic inflammation (22). Treatment with TGFβ1 was found to suppress the increase in the number of circulating leukocytes, suggesting that the inhibition of leukocytosis may be important in preventing the arthritic condition. This effect was not noted during the acute phase, but was observed consistently in the chronic phase and was dependent on the amount of TGFβ1 administered. The inhibition of SCW-induced leukocytosis may be due to a decrease in the proliferation of hematopoietic precursor cells in the bone marrow. Administration of TGFβ1 to mice via the femoral artery has recently been demonstrated to cause the partial inhibition of bone marrow proliferation (9). Several in vitro studies support this observation (6–8). Thus, the limited recruitment of inflammatory cells into the joint may be due, in part, to a lower number of circulating WBCs.

In addition to reduced numbers of circulating WBCs, chemotaxis of inflammatory cells into the joint may also be altered by the treatment with TGFβ1. TGFβ has been identified as a potent monocyte chemotactic factor (23), and its importance as a synovial chemotactic factor was shown in a recent study in which a local injection of TGFβ into normal rat joints caused an immediate influx of inflammatory cells (19). Hence, a high concentration of circulating TGFβ may effectively eliminate a

synovium-centered TGFβ gradient, inhibiting TGFβ-mediated inflammatory cell influx. This mechanism is consistent with the decreased number of inflammatory cells found in the joint. In addition to changes in the TGFβ gradient, systemic administration of TGFβ may alter inflammatory cell expression of TGFβ receptors. Exposure of circulating human monocytes to TGFβ effectively downregulates TGFβ receptor expression (Brandes and Wahl, in preparation), in contrast to the lack of ligand-induced receptor downregulation observed in other cell populations (24). Thus, a diminished pool of circulating WBC and a decreased chemotactic response to TGFβ might effectively restrict synovial inflammatory events dependent on cell recruitment. TGFβ has also been shown to inhibit neutrophil adhesion to endothelial cells, the event preceding cell migration into the tissue (25). Systemic administration of TGFβ1 may decrease blood cell adhesion to the endothelium, thus also contributing to the limited inflammatory cell recruitment into the joint. Those inflammatory cells that are able to infiltrate the tissue may also exhibit suppressed function, since TGFβ has recently been shown to decrease IL-1 receptor expression (26) and the production of superoxide radical both in vitro (27) and in vivo (28; and Allen, Brandes, Ogawa, and Wahl, manuscript in preparation).

Additional effects of TGFβ identified using in vitro systems may also contribute to the ability of TGFβ to ameliorate SCW arthritis. For example, TGFβ inhibits IL-1-induced chondrocyte protease activity and cartilage proteoglycan degradation (29). Furthermore, TGFβ inhibits the formation of osteoclast-like cells in long term human marrow cultures (30) and inhibits bone resorption (31). Thus, systemic TGFβ1 administration may act directly to contain the bone and cartilage erosion, as well as indirectly by suppressing inflammatory cell infiltration. Studies are in progress to document the contribution of these and/or other pathways relevant to TGFβ suppression of erosive arthritis.

Surprisingly, there was no evidence of abnormalities in the number or in vitro proliferative behavior of cells isolated from the spleen and peripheral blood of TGFβ1-treated control animals. Moreover, TGFβ did not further inhibit the already suppressed proliferation of spleen cells and PBMC from SCW-injected animals. Thus, either generalized immunosuppression does not occur, or it is reversible. In addition, treatment of control animals with TGFβ1 did not affect their normal weight gain or cause noticeable pathologies. This is in contrast to previous studies in mice injected subcutaneously with > 20 μg of TGFβ1 per day. The mice developed anemia, thrombocytopenia, increased white cell count (32), and 20–30% loss in body weight during the 14-d dosing regimen (J. A. Carlino, personal communication).

TGFβ1 was shown in this study to effectively inhibit the development of an induced arthritic condition in rats, likely via its immunoregulatory effects (33) and its inhibition of connective tissue degradation (34). Although TGFβ has been administered in vivo for acute inflammatory events (28, 35), this is the first study to address the therapeutic potential of TGFβ for the treatment of chronic inflammatory diseases. Our data suggest that TGFβ is effective in controlling the disease process, while producing minimal side effects despite its presence in non-physiological quantities. If TGFβ can successfully treat immune system disorders, and do so with limited toxic effects, then it may warrant consideration as a valuable therapeutic agent.

Acknowledgments

The authors thank Dr. Uwe Mai and Dr. Nancy McCartney-Francis for helpful discussions.

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