

BACTERIAL CELL WALL-INDUCED IMMUNOSUPPRESSION

Role of Transforming Growth Factor β

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A single intraperitoneal injection of bacterial cell walls derived from group A streptococci (SCW)¹ into susceptible rats results in the development of acute and chronic erosive polyarthritis (1, 2). The pathology of the lesion leading to joint destruction has been shown to be a T cell-dependent, cell-mediated response to the deposition of the group A streptococcal peptidoglycan-polysaccharide polymers in the synovium (3-5). Furthermore, these chronic joint lesions share many common histopathologic, radiologic, and clinical features with human rheumatoid arthritis (6). Another apparent shared feature in patients with rheumatoid arthritis and rodents with SCW-induced arthritis is impaired immunoregulation and suppression of the immune system. Patients with arthritis frequently exhibit suppressed lymphoid cell functions (6-10), and depressed lymphoid cell activity has also been reported in SCW-treated rats (2, 11, 12). The mechanism of aberrant immune responsiveness associated with these chronic inflammatory lesions is unknown, although adherent cells have been implicated (2, 10) and deficient IL-2 production has also been reported (11).

Because of the potential importance of impaired immunoregulation in the evolution of chronic inflammatory lesions such as arthritis, we have used the experimental model of SCW-induced chronic inflammation to explore the cellular and molecular pathways of suppressed immune responsiveness. In this study, we document a role for the adherent macrophage population in mediating the suppressed lymphoproliferative responses characteristic of the spleens of SCW-treated Lewis (LEW) rats. Furthermore, this suppression is mediated by a soluble suppressor factor released by SCW-activated macrophages within the spleen. Subsequent studies to characterize this suppressor activity revealed it to be consistent with the polypeptide, transforming growth factor β (TGF- β).

Materials and Methods

Animals. Specific pathogen-free inbred Lewis (LEW) female rats (100 g) (Charles River Breeding Laboratories, Inc., Wilmington, MA) were injected with preparations of peptidoglycan-polysaccharide fragments from SCW prepared as described (2, 3). A sterile aqueous suspension of sonicated SCW in PBS, pH 7.4 (National Institutes of Health Media

¹ *Abbreviations used in this paper:* LEW, Lewis; SCW, group A streptococcal cell walls; TGF- β , transforming growth factor β .

Unit), was injected intraperitoneally at an optimal dose equivalent to 30 μ g of cell wall rhamnose per gram body weight. Control rats received PBS only, and all animals were monitored for the development of clinically identifiable arthritis.

Spleen Cell Proliferation and Supernatant Production. Spleens were excised at various intervals after SCW injection and from control animals and single cell suspensions prepared (13). Data is presented from spleens obtained 3 wk after SCW administration unless otherwise indicated. Proliferation was determined by culturing 2×10^5 cells/200 μ l DMEM containing 50 μ g/ml gentamicin, 2 mM glutamine, 5×10^{-5} M 2-ME, and 1% normal rat serum in microtiter wells with PHA (Burroughs Wellcome Co., Greenville, NC) or Con A (Calbiochem-Behring Corp., San Diego, CA) (13). After 68 h of culture, the cells were pulsed 4 h with 0.5 μ Ci/well of [3 H]thymidine ([3 H]TdR; sp act, 6.7 Ci/mmol, Becton Dickinson & Co., Mountain View, CA). The cultures were harvested with an automated harvester (PhD., Cambridge Technology, Inc., Cambridge, MA) and processed for determination of [3 H]TdR incorporation. All data are means of triplicates in which the SE were routinely <10% of the mean. For coculture experiments, spleen cells were separated into adherent and nonadherent populations by adhering the spleen cells for 2–4 h followed by repeated washing. The recovered nonadherent cells were resuspended in DMEM, counted, and cultured in the presence or absence of adherent cell populations (>90% macrophages by nonspecific esterase staining).

Cytokine-enriched supernatants were generated by incubating 2×10^6 unseparated spleen cells, adherent cells, or nonadherent cells in 1 ml serum-free DMEM without stimuli or with 5 μ g/ml Con A, 5 μ g/ml PHA, or 10 μ g/ml LPS (055:B5; Difco Laboratories Inc., Detroit, MI) for 24–48 h. In certain experiments, culture supernatants were preincubated with a previously determined optimal concentration of a rabbit IgG antibody that neutralizes both TGF- β 1 and TGF- β 2 (R & D Systems, Inc., Minneapolis, MN). Dependent upon the assay, 10–50 μ g of IgG neutralize the activity of 1 ng/ml of TGF- β , and therefore, we routinely added 5 μ g IgG in 5 μ l PBS to 100 μ l of supernatant. Equivalent concentrations of normal rabbit IgG (kind gift of Dr. R. Mage, National Institute of Allergy and Infectious Diseases) were added to parallel aliquots of supernatant as a control.

Identification of Cell Subpopulations, SCW, and TGF- β in Rat Spleens. Spleens were flash frozen in O. C. T. Compound (Miles Scientific Div., Naperville, IL), sectioned, fixed in acetone, and preincubated for 10 min with a 1:200 dilution of horse serum and rat serum (3). The following mAbs were used in combination with an immunoperoxidase staining technique (ABC VectaStain Kit; Vector Laboratories, Inc., Burlingame, CA) for identification of spleen mononuclear cell subpopulations: W3/13 (pan T lymphocytes), OX19 (pan T lymphocytes), W3/25 (CD4 helper/inducer lymphocytes), OX8 (CD8, suppressor/cytotoxic lymphocytes), OX-43 (macrophages), and Ia (Accurate Chemical & Scientific Corp., Westbury, NY). Additional spleen sections were stained with a rabbit antibody to the group A-specific SCW (3) or with an antibody to TGF- β prepared against a synthetic polypeptide identical to NH₂-terminal residues 1–30 of TGF- β (14). Control sections were stained as above without the primary antibody or with an irrelevant mAb.

In additional studies, single cell suspensions from spleens of SCW-treated and control animals were incubated at 4°C (5×10^3 cells/50 μ l staining buffer containing 0.1% NaN₃ and 2% FCS) with 10 μ l of a 1:500 dilution of the above antibodies, washed, and then incubated with a FITC-goat (Fab)₂ anti-mouse Ig antibody (Tago Inc., Burlingame, CA). The cells were washed and suspended in 2% paraformaldehyde before analysis by flow microfluorometry (FACStar; Becton Dickinson & Co., Sunnyvale, CA) as described (15).

IL-2 Assay. Supernatants were assayed for IL-2 activity by their ability to support the growth of the murine IL-2-dependent CTL-L line (13). The CTL-L cells were cultured in the presence of threefold dilutions of the spleen cell supernatants in parallel with an IL-2 standard containing 100 U/ml for 48 h. The triplicate cultures were pulsed with 0.5 μ Ci [3 H]TdR (sp act, 1.9 Ci/mmol) for 4 h, and the incorporated radioactivity was determined. The IL-2 activity in test samples is presented as units of IL-2 based on comparison with the titration curve of the IL-2 standard.

IL-1 Assay. Supernatants (twofold dilutions) were assayed for IL-1 activity in cultures of thymocytes from 6-wk-old C3H/HeJ mice with suboptimal concentrations of PHA (13). During the final 5 h of incubation, the cultures were pulsed with 0.5 μ Ci/well [3 H]TdR, the incor-

porated radioactivity was determined, and the data were transformed into units by comparison to the IL-1 standard (Genzyme, Boston, MA) containing 100 U/ml.

Northern Blot Analysis. Total RNA was isolated from spleens obtained from SCW-injected or control animals 12 h to 3 wk after SCW administration. Total RNA was extracted with guanidine thiocyanate as described (16). The RNA samples (10.0 μ g) were subjected to electrophoresis in a formaldehyde-containing agarose gel and transferred to nitrocellulose paper. The filters containing spleen cell RNA were prehybridized for at least 4 h and then hybridized overnight at 42°C with a 32 P-labeled Eco R1 insert of TGF- β obtained from Genentech (S. San Francisco, CA). The filters were exposed to Kodak XAR-5 film with intensifier screens at -70°C for 4-18 h. Relative amounts of mRNA present on the filter were monitored by stripping the blots and then hybridizing with a 32 P-labeled plasmid encoding rat glyceraldehyde-3-phosphate-dehydrogenase (17).

Results

Deficient Lymphoproliferative Responses in SCW-injected LEW Rats. Spleen cells obtained from LEW rats 3 wk after a single intraperitoneal injection of SCW were compared with age-matched control LEW rat spleen cells for their ability to proliferate in response to T cell mitogens. As evident in Fig. 1, spleen cells from the SCW-injected animals did not proliferate to either Con A or PHA in contrast to similar spleen cell cultures from LEW control animals. This deficient lymphoproliferative response was independent of antigen, mitogen, dose, cell concentration, duration of culture, or differences in cell viability. Evaluation of lymphocyte function at various time points after SCW administration revealed that within 18 h the proliferative response was suppressed. This immune-deficient state continued through the development of acute arthritic symptoms (days 3-5) and persisted during the evolution of the chronic erosive joint lesions (>3 wk) (Fig. 2). Suppressed lymphocyte DNA synthesis in response to *in vitro* stimulation was also apparent in cells obtained

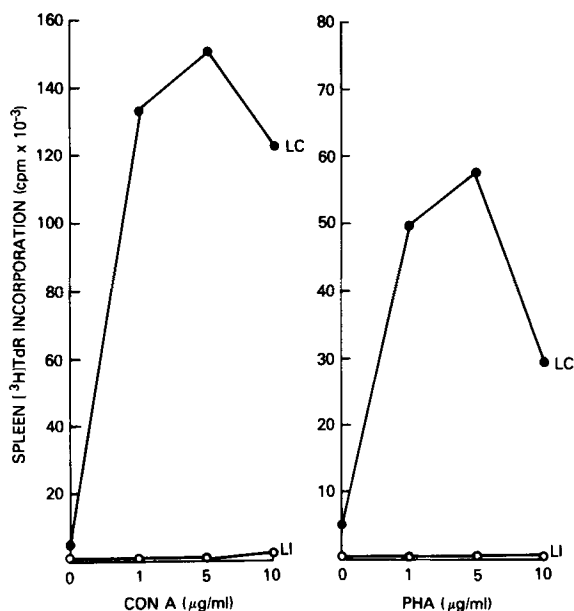


FIGURE 1. Suppressed lymphocyte mitogenesis after SCW injection. Spleen cells prepared from LEW rats 3 wk after a single intraperitoneal injection of SCW (LI) or control animals (LC) were cultured with 0-10 μ g/ml Con A or PHA for 72 h. The triplicate cultures were pulsed with [3 H]TdR and processed for determination of mitogenic activity. Data are representative of 10 experiments.

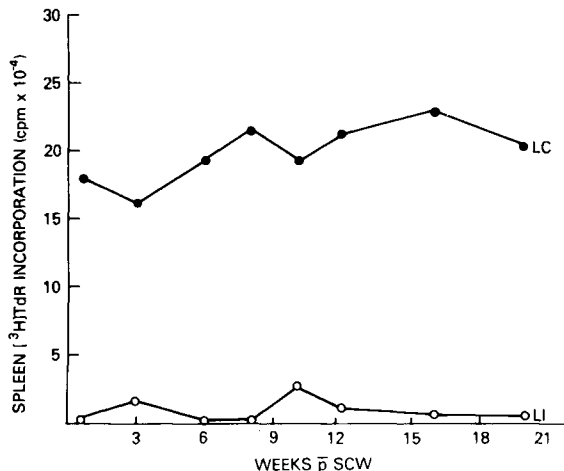


FIGURE 2. Kinetics of SCW-induced immunosuppression. Spleen cells were obtained from animals 18 h to 21 wk after a single intraperitoneal injection of SCW (LI) or from age-matched control animals (LC) and challenged with an optimal dose of Con A (5 μ g/ml). Data are representative of four to eight animals at each time interval.

from draining lymph nodes and in PBMC from the SCW-injected animals (data not shown).

Cytokine Production by Spleen Cells from Normal and SCW-treated LEW Rats. To determine whether the spleen cell dysfunction was a consequence of a deficiency in the generation of cytokines that promote mitogenesis, IL-1 and IL-2 levels were quantitated in the spleen cell culture supernatants (Fig. 3). Levels of IL-2 produced by spleen cell cultures from SCW-treated animals in response to Con A challenge were frequently \sim 50% of those produced by control spleen cells under similar conditions (Fig. 3 A) as recently reported (11). However, the production of IL-2 in response to PHA stimulation was only marginally reduced in the SCW-injected rat spleen cell cultures. These data suggested that although reduced IL-2 levels might contribute to the decreased proliferative response, IL-2 deficiency likely could not be considered the sole mechanism responsible for the immunosuppression. Furthermore, the addition of exogenous IL-2 (up to 100 U/ml) was ineffective in reversing the depressed mitogenic response (data not shown).

Similarly, IL-1 production by cultures of spleen cells from SCW-treated rats stimulated with LPS in vitro was not significantly diminished compared with controls (Fig. 3 B), and in adherent spleen cell-enriched populations the IL-1 levels were frequently found to be elevated. IL-1 added to spleen cell cultures from SCW-treated rats did not augment the mitogen-induced proliferation. In further studies to evaluate cytokine production, both granulocyte-macrophage colony stimulating factor and IL-3 synthesis were not significantly different between mitogen-stimulated control and SCW spleen cell cultures (data not shown). Thus, these data collectively demonstrate that a deficiency in cytokine production is probably not the primary mechanism responsible for the suppressed proliferative responses, and that additional mechanisms whereby SCW mediates immunosuppression must be operative.

Role of Adherent Cells in Mediating Immunosuppression. Since a deficiency in mononuclear cell-derived cytokines necessary for cell growth could not account for the lack of mitogenesis, spleen cells from LEW-injected and LEW control animals were compared for aberrations in cellular subsets which might explain the nature of the

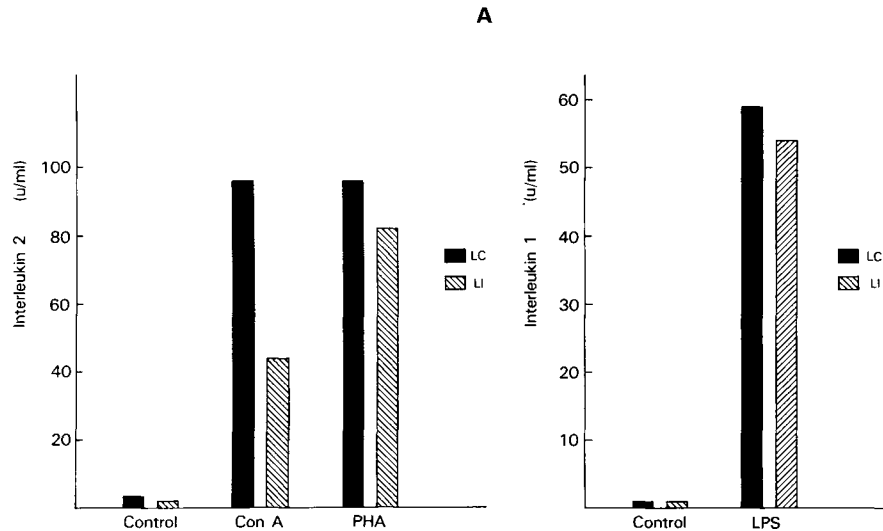


FIGURE 3. IL synthesis by control and SCW-injected spleen cells. Spleen cells were cultured at 2×10^6 /ml in the presence or absence of PHA (5 μ g/ml), Con A (5 μ g/ml), or LPS (10 μ g/ml) for 48 h. (A) Supernatants were harvested and assayed for IL-2 on the CTL-L cell line as described in Materials and Methods. Data represent mean U/ml IL-2 from six different animals. (B) IL-1 from LPS-stimulated culture supernatants was quantitated in the thymocyte costimulator assay.

suppression. Although an increase in the T cell suppressor population might be anticipated, we and others (11) could not demonstrate an increase in the percentage of cells that stained positively for the CD8 marker in immunocytochemical analysis of spleen tissue sections or by FACS analysis ($20.5 \pm 7.2\%$ CD8⁺ in control vs. $14.5 \pm 6.01\%$ in 6 SCW injected). CD4⁺ helper T cells transiently increased in percentage, although not significantly above controls. The only consistent alteration in subsets observed was an increase in OX43⁺ staining, which identifies certain monocyte-macrophage populations (18). Control spleens contained 2–5% OX43⁺ cells, whereas 5–25% OX43⁺ cells were identified in the spleens of SCW-injected animals depending on the interval after treatment. To determine if this increase in monocyte-macrophages was contributing to the suppressed T cell response, the spleen cells from control and SCW-treated animals were separated into adherent and nonadherent cell populations before culture with mitogens. Depletion of adherent cells from LEW control spleen cell populations resulted in diminished mitogen-induced DNA synthesis, likely due to loss of accessory cell function (Fig. 4 A and B). In contrast, removal of adherent cells from the SCW-injected spleen cell preparations resulted in dramatically enhanced T cell proliferation to Con A (Fig. 4 A) and PHA (Fig. 4 B) to normal or near normal levels. These data clearly indicated that the defect in T cell proliferation was reversible and also, that it was mediated by adherent cells. Furthermore, T lymphocytes themselves are likely not defective in IL-2 synthesis, IL-2-R expression, or other parameters of growth since the T cells functioned normally after removal of adherent cells.

To further document the role of the adherent spleen cells (>90% macrophages)

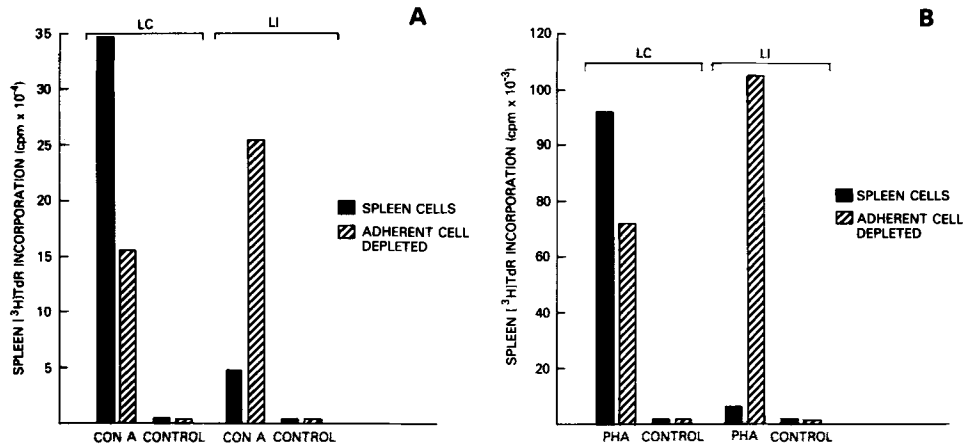


FIGURE 4. Restoration of lymphocyte mitogenesis by adherent cell depletion. Spleen cells (2×10^7 /ml) from control (LC) and SCW-injected (LI) animals were cultured for 2-4 h, and the nonadherent cells were harvested, counted, and cultured (2×10^5 /200 μ l) in the presence or absence of Con A (5 μ g/ml) or PHA (5 μ g/ml) for 72 h. Mitogenic activity in the triplicate cultures was determined after a 4-h pulse with [³H]TdR. Data are representative of four to six experiments.

from the SCW-treated animals in inhibiting lymphocyte proliferation, adherent cells from SCW-injected animals were cocultured with nonadherent spleen cells from control animals (Fig. 5 A and B, *LI adh* and *LC nonadh*). Under these conditions, marked suppression of the mitogen response of the control lymphocytes was observed. In contrast, control adherent cell populations cocultured with control nonadherent cells (Fig. 5, *LC nonadh* and *LC adh*) demonstrated vigorous proliferation to both mitogens. Cultures containing adherent cells from SCW-treated animals and nonadherent spleen cells from the same animals (Fig. 5, *LI adh* and *LI nonadh*, respectively) exhibited no proliferation in response to Con A or PHA (Fig. 5, A and B). After in vivo ex-

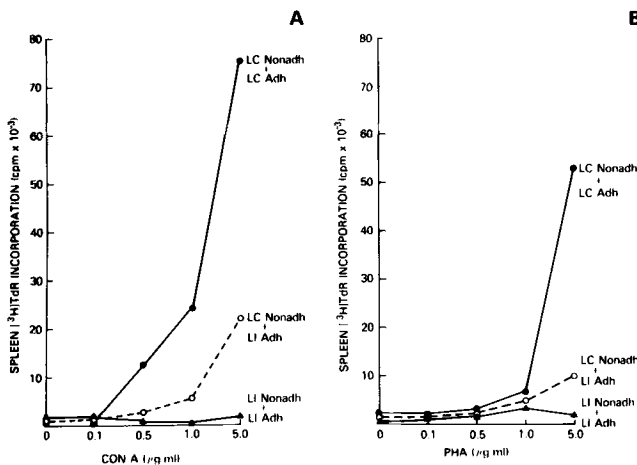


FIGURE 5. Effect of adherent cell populations on spleen cell mitogenic activity. Spleen cells (2×10^5 /200 μ l) from control (LC) or SCW injected (LI) animals were cultured for 2-4 h in microtiter wells. The non-adherent cells were recovered and cocultured with adherent cells from LC or LI cultures for the indicated concentrations of Con A or PHA. Mitogenic activity in the triplicate cultures was determined after a 4-h pulse with [³H]TdR. Data are representative of four to six experiments.

posure to SCW, adherent spleen macrophages appear to exhibit profound inhibitory effects on T lymphocyte mitogenic activity.

Mechanism of Adherent Cell Suppression. Efforts were subsequently directed at defining the mechanisms whereby the adherent macrophages suppressed lymphocyte proliferation. The possibility of a soluble suppressor factor was explored by culturing spleen cell populations from control and SCW-injected rats without any exogenous stimuli for 24–48 h, collecting the cell-free supernatants, and then adding these supernatants to cultures of control spleen cells in the presence of Con A. The addition of supernatants obtained from LC spleen cultures augmented the mitogenic response of Con A-stimulated control spleen cells (Fig. 6). In contrast, supernatants obtained from parallel SCW-injected rat spleen cell cultures suppressed the normal mitogenic response from 10 to 80%, dependent upon concentration and kinetics (Fig. 6, *LI 1–3*). These data supported the notion that a macrophage-dependent soluble suppressor molecule(s) was generated after exposure to the bacterial cell walls *in vivo*.

Initial attempts to define this suppressor molecule involved the addition of inhibitors of several previously defined macrophage suppressor molecules including prostaglandins (19) and reactive oxygen intermediates (20). Although SCW added directly to spleen cell and/or macrophage cultures induces PGE₂ synthesis and O₂⁻ generation (Feldman et al., manuscript in preparation), the addition of indomethacin (10⁻⁶ M), superoxide dismutase (up to 600 µg/ml) and/or catalase (up to 10,000 U/ml) to the suppressed spleen cell cultures was ineffective in reversing the diminished mitogenic response (data not shown).

Role of TGF-β in Spleen Cell Suppression. Macrophages produce additional suppressor molecules including TGF-β (21), which we have recently shown to be an extremely potent inhibitor of IL1-dependent thymocyte proliferation (22). To deter-

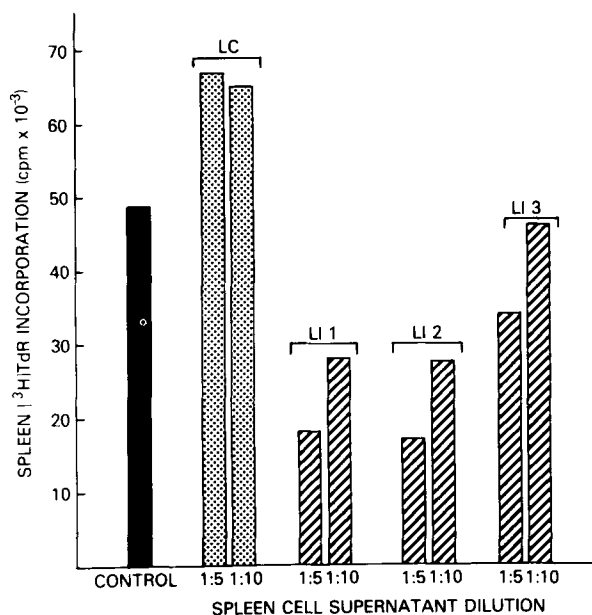


FIGURE 6. Inhibition of lymphocyte proliferation by soluble suppressor factor. Spleen cells (4×10^6 /ml) from control (*LC*) or three different SCW-injected (*LI 1–3*) animals were cultured without stimulation *in vitro* for 24 h, and the cell-free supernatants were collected. These supernatants (unconcentrated) were diluted as indicated into control spleen cell cultures challenged with Con A (5 µg/ml) for 72 h. Mitogenic activity was determined after a 4-h pulse with [³H]Tdr.

mine whether the soluble suppressor molecule(s) produced by the macrophages might be TGF- β , the supernatants with suppressor activity were treated with a rabbit antibody to TGF- β (R & D Systems, Inc.) or a control rabbit IgG preparation before their addition to normal spleen cell cultures. This antibody neutralizes both TGF- β 1 and TGF- β 2. As is evident in Table I, pretreatment of the supernatants from spleen cell cultures obtained from SCW-treated rats with optimal concentrations (50 μ g IgG/ml) of the anti-TGF- β significantly blocked the suppressor activity of these supernatants causing a 10-fold increase in proliferation. The control serum was ineffective in reversing the inhibitory activity. Neutralization of the majority of the suppressor activity with an antibody to TGF- β suggested coidentity with TGF- β , although in several experiments suppressor activity could not be completely blocked even at higher concentrations of the antibody. These data suggest that other suppressor molecules in the spleen cell culture supernatants may also contribute to the immune suppression. Furthermore, addition of rTGF- β (kindly provided by Genentech) to control spleen cell cultures in nanogram quantities effectively reproduced the suppression of normal spleen cell proliferative responses to T cell mitogen (Table II) caused by the suppressor supernatants.

Gene Expression for TGF- β in Control and SCW Spleens. To determine whether the bacterial cell walls induced gene expression for TGF- β in the spleens and/or macrophages of treated animals, spleens were obtained from 12 h to 3 wk after the injection of SCW and processed for RNA isolation. When equivalent amounts of RNA from control and SCW-treated spleens were hybridized by Northern analysis with a cDNA probe for TGF- β , mRNA levels for TGF- β were not significantly different in control or SCW-treated spleen cells whether evaluated at 12 h or at 3 wk after SCW treatment (Fig. 7). Furthermore, the same pattern of TGF- β mRNA expression was observed when adherent spleen macrophage mRNA was probed for TGF- β (data not shown). These observations are consistent with recent reports that macrophages constitutively express mRNA for TGF- β and that activation of the cells does not increase transcription, but rather induces the synthesis and secretion of TGF- β (21).

TABLE I
Antibody to TGF- β Blocks Suppressor Activity

Supernatant	Lymphocyte proliferation ([³ H]TdR incorporation)	
	Control	Con A
	<i>cpm</i>	
None	1,032	284,593
Sup + PBS	84	18,506
Sup + NRS	77	13,898
Sup + Anti-TGF- β	319	139,954

24-h supernatants (Sup) (100 μ l) from SCW-treated rat spleen cell cultures were incubated with 5 μ l PBS, 5 μ l rabbit anti-TGF- β antibody, or with an equivalent amount of normal rabbit IgG (NRS) for 30 min before being added (40 μ l) to normal spleen cell cultures with or without 5 μ g/ml Con A. Normal spleen cell cultures without any supernatant (none) served as positive controls. After 72 h, the triplicate cultures were pulsed with [³H]TdR and processed for determination of incorporated [³H]TdR.

TABLE II
Suppression of Spleen Cell Proliferation by rTGF- β

TGF- β ng/ml	Lymphocyte Proliferation ([³ H]TdR Incorporation [Con A])		
	0 μ g/ml	1 μ g/ml	5 μ g/ml
0	1,720	46,380	24,446
0.1	1,583	16,560	16,056
1.0	400	8,883	6,710

Spleen cells (2×10^5) were stimulated with 0, 1, or 5 μ g/ml Con A in the presence or absence of recombinant TGF- β at the indicated concentrations. After 72 h, the cultures were pulsed with [³H]TdR and processed for determination of incorporated [³H]TdR. Data represent the mean of triplicate cultures.

In Situ Localization of TGF- β . By immunohistochemical localization of TGF- β , however, it became clear that although gene expression for TGF- β might not be different in control and SCW-injected spleens, production of the TGF- β gene product was clearly different in the injected and noninjected spleens. As shown in Fig. 8 C, tissue sections of spleens from SCW-treated animals demonstrated substantial immunoperoxidase staining with the antibody to TGF- β in contrast to control spleens (Fig. 8 F) that were essentially negative for TGF- β . Furthermore, the localization of the TGF- β appears to coincide with the phagocytic cells in the spleen. Serial sec-

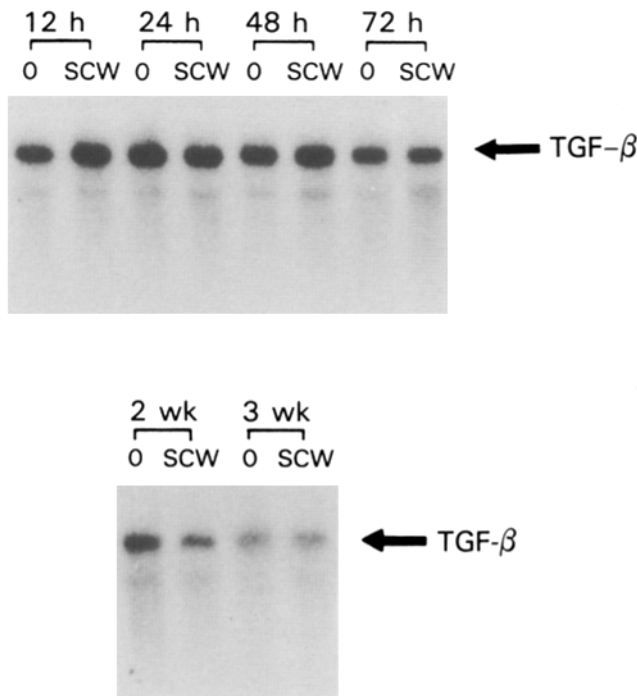


FIGURE 7. Gene expression for TGF- β in spleens from control and SCW-injected animals. Spleens were obtained 12 h to 3 wk after an intraperitoneal injection of SCW (SCW) or PBS (0) and processed for mRNA isolation. Equivalent amounts of RNA on nitrocellulose filters were hybridized with a ³²P-labeled probe for TGF- β . The filters were exposed to Kodak XAR-5 film with intensifier screens at -70°C .

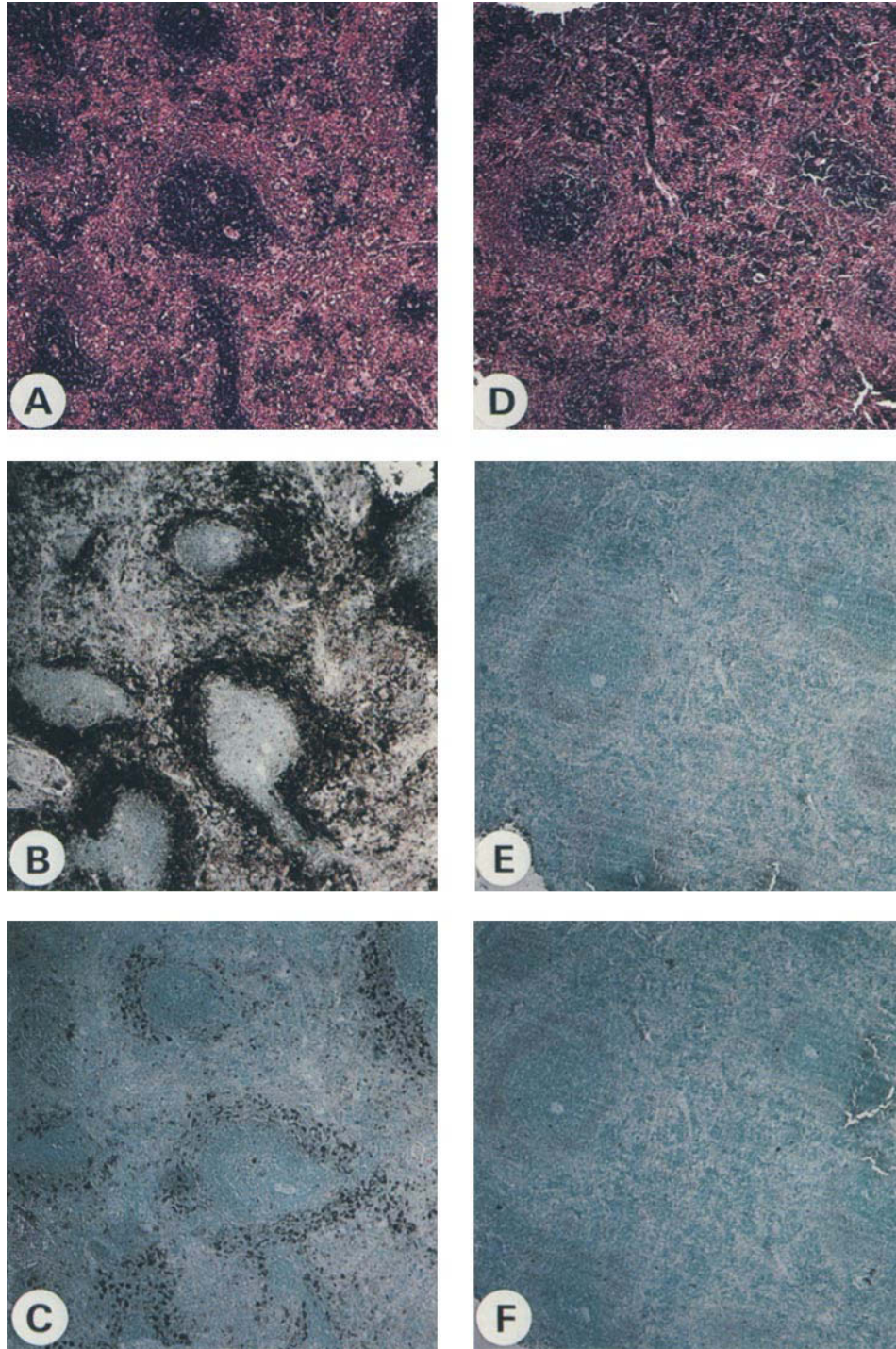


FIGURE 8. Immunocytochemical localization of SCW and TGF- β in spleen sections from control and SCW-injected animals. Representative serial spleen sections from 3-d SCW-injected (*A-C*) and control (*D-F*) animals were stained with hematoxylin and eosin (*A* and *D*); with an antibody to SCW (*B* and *E*); or with an antibody to TGF- β prepared against NH₂-terminal residues 1-30 of TGF- β (*C* and *F*) by the immunoperoxidase technique. The black precipitate identifies the reaction product. ($\times 100$).

tions of the spleens from SCW-injected and control animals were stained in parallel with an antibody to SCW (Fig. 8, *B* and *E*). Areas containing cells that have phagocytized SCW also contain cells that are positive for TGF- β . At higher magnification (Fig. 9), the colocalization of cells containing SCW and those positive for TGF- β is more apparent. Interestingly, the predominantly T cell-populated regions stained minimally or not at all with the antibody for TGF- β . These in situ data support the in vitro evidence that the macrophages are the primary source of the suppressor activity and that this suppressor activity can be attributed, at least in part, to TGF- β .

Discussion

TGF- β has recently been shown to be a bifunctional regulator of various cellular and immunologic functions (23). TGF- β can both activate (16) and inhibit (22, 24–28) immune cell function. While TGF- β has been shown to be a potent immunosuppressive agent in vitro, no demonstration of such a role has previously been documented in vivo. In this study, we demonstrate that SCW-activated phagocytic cells exert a profound long-lasting immunosuppressive effect on lymphocyte function in the spleens of SCW-injected animals. There was no significant change in the percentage of CD8⁺ and CD4⁺ T lymphocytes in the spleens of the SCW-treated animals. The only change in the cellular composition of the spleens was an increase in macrophage representation. By various coculture and depletion techniques, it became ap-

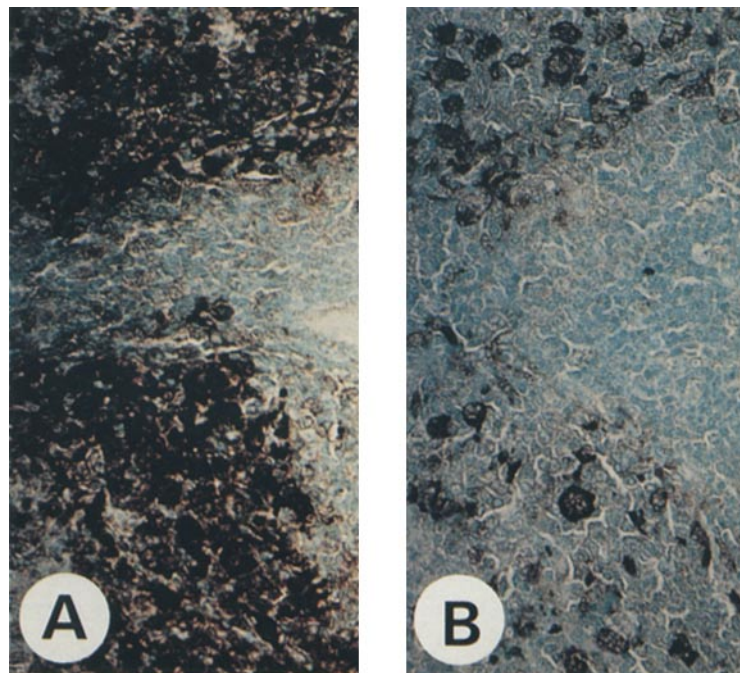


FIGURE 9. Immunocytochemical localization of SCW and TGF- β in spleen sections. Spleen sections from SCW-injected animals stained as above with antibodies to SCW (*A*) and TGF- β (*B*). (\times 300).

parent that the adherent cells were involved in the regulation of the SCW-induced state of immunodeficiency. In culture, the adherent cells (>90% macrophages) released a soluble molecule into the culture supernatants, which effectively suppressed normal lymphocyte mitogenesis. Furthermore, this suppressor activity could be neutralized with an antibody to TGF- β .

Further documentation of a macrophage-TGF- β pathway for immune suppression was provided by in situ localization of TGF- β in association with the phagocytic cells of the spleens of SCW-treated animals. Interestingly, the mechanism of immunosuppression mediated by the SCW-activated phagocytic cells appears to be similar to previous reports on the mechanism of TGF- β -inhibited mitogenesis in vitro (22). Although TGF- β blocked DNA synthesis, it did not block the synthesis of lymphokines such as IL-2 nor the expression of IL-2-R on the mitogen-activated T cells (22). Lymphocytes obtained from the spleens of SCW-injected animals also did not proliferate in response to mitogens, yet generated IL-2, GM-CSF, and IL-3 consistent with a TGF- β -mediated block in DNA synthesis, but not cytokine production. The previously reported deficit in IL-2 production in such cultures (11) may be related to a decreased number of IL-2-producing cells rather than a defective synthesis of IL-2, since reduced IL-2 production is reversible after removal of adherent suppressor cells. IL-1 production also was not decreased and was often increased above the controls as might be anticipated, since TGF- β has been shown to induce the synthesis and secretion of IL-1 (16, 22).

Activated macrophages have frequently been associated with various forms of immunodeficiency, and a number of macrophage-derived suppressor molecules have been reported including prostaglandins (19), reactive oxygen intermediates (20), and a variety of uncharacterized factors produced in response to virus, mycobacteria, and other stimuli (29-33). The peptidoglycan-polysaccharide polymers of SCW stimulate human (Allen et al., manuscript in preparation) and rat (Feldman et al. manuscript in preparation) macrophages to generate PGE₂, O₂⁻ and monokines including TGF- β . The mechanisms of interaction between these and other macrophage-derived immunoregulatory molecules in vivo to promote and/or control an inflammatory response are unclear. However, in the spleens of SCW-injected rats, it appears that the balance of factors favors the ability of TGF- β to down-regulate lymphoproliferative responses.

Many chronic inflammatory conditions, including rheumatoid arthritis (6-10), leprosy (34), and tuberculosis (35), are associated with deficient cell-mediated immune responses. These abnormalities in immunoregulation have often been attributed to an active, adherent, suppressor cell population (10, 36, 37). Although there is, as yet, no evidence linking TGF- β to the aberrant immune responses in any of these immunosuppressed conditions, recent evidence from our laboratory suggests that TGF- β may contribute to the abnormalities in immunoregulation apparent in the synovium of patients with rheumatoid arthritis (Wahl et al., manuscript submitted for publication). The unique ability of TGF- β to augment certain aspects of the immune response while dampening others (16, 22, 23) suggests that it may play an important immunoregulatory role in chronic inflammatory disorders.

Summary

Group A streptococcal cell wall (SCW)-injected rats exhibit a profound immunosup-

pression that persists for months after the initial intraperitoneal injection of SCW. The goal of this study was to determine the mechanisms for the suppressed T lymphocyte proliferative responses in this experimental model of chronic inflammation. When spleen cell preparations were depleted of adherent cells, restoration of T cell proliferative responses to Con A and PHA occurred, implicating adherent macrophages in the regulation of immunosuppression. Furthermore, macrophages from SCW-treated animals, when cocultured with normal spleen cells in the presence of Con A or PHA, effectively inhibited the proliferative response. Supernatants from suppressed spleen cell cultures were found to inhibit normal T cell mitogenesis. Taken together, these results implicated a soluble macrophage-derived suppressor factor in the down regulation of T cell proliferation after exposure to SCW *in vivo*. Subsequent *in vitro* studies to identify this suppressor molecule(s) revealed the activity to be indistinguishable from the polypeptide transforming growth factor β (TGF- β). Furthermore, TGF- β was identified by immunolocalization within the spleens of SCW-injected animals. The cells within the spleen that stained positively for TGF- β were phagocytic cells that had ingested, and were presumably activated by, the SCW. These studies document that TGF- β , previously shown to be a potent immunosuppressive agent *in vitro*, also effectively inhibits immune function in chronic inflammatory lesions *in vivo*.

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