JCI The Journal of Clinical Investigation

Intracellular demonstration of active TGFbeta1 in B cells and plasma cells of autoimmune mice. IgG-bound TGFbeta1 suppresses neutrophil function and host defense against Staphylococcus aureus infection.

T E Caver, … , L I Gold, H D Gresham

J Clin Invest. 1996;98(11):2496-2506. https://doi.org/10.1172/JCI119068.

Research Article

Infection remains a leading cause of morbidity and mortality in patients with SLE. To investigate this, previously we assessed the host defense status of autoimmune MRL/lpr mice and found that elaboration of active TGFbeta suppressed neutrophil function and decreased survival in response to Staphylococcus aureus infection. The purpose of the present work was to elucidate the molecular form and the cellular source of the active TGFbeta involved. Here, we report for the first time that TGFbeta1 is found in the active form inside B cells and plasma cells and that it circulates in the plasma complexed with IgG in two murine models of systemic autoimmunity and in some patients with SLE. IgG-bound active TGFbeta1 is many times more potent than uncomplexed active TGFbeta1 for suppression of neutrophil function in vitro and host defense against S. aureus infection in vivo. These data indicate that TGFbeta1 is in the active form inside B cells and plasma cells, that the formation of a complex of IgG and active TGFbeta1 is greatly accelerated in autoimmunity, and that this complex is extremely potent for suppression of PMN function and host defense against bacterial infection.

Find the latest version:

https://jci.me/119068/pdf

Intracellular Demonstration of Active TGFβ1 in B Cells and Plasma Cells of Autoimmune Mice

IgG-Bound TGFβ1 Suppresses Neutrophil Function and Host Defense against *Staphylococcus aureus* Infection

Tony E. Caver,*‡§ Frank X. O'Sullivan,*‖ Leslie I. Gold,¶ and Hattie D. Gresham*‡§

**Research Service, Harry S. Truman VA Medical Center, Columbia, Missouri 65201; ‡Department of Pharmacology, §Department of Molecular Microbiology and Immunology, and ‖Department of Medicine, University of Missouri-Columbia, Columbia, Missouri 65212; and ¶Department of Pathology, New York University Medical Center, New York 10016*

Abstract

Infection remains a leading cause of morbidity and mortality in patients with SLE. To investigate this, previously we assessed the host defense status of autoimmune MRL/lpr mice and found that elaboration of active TGFβ suppressed neutrophil function and decreased survival in response to *Staphylococcus aureus* infection. The purpose of the present work was to elucidate the molecular form and the cellular source of the active TGFβ involved. Here, we report for the first time that TGFβ1 is found in the active form inside B cells and plasma cells and that it circulates in the plasma complexed with IgG in two murine models of systemic autoimmunity and in some patients with SLE. IgG-bound active TGFβ1 is many times more potent than uncomplexed active TGFβ1 for suppression of neutrophil function in vitro and host defense against *S. aureus* infection in vivo. These data indicate that TGFβ1 is in the active form inside B cells and plasma cells, that the formation of a complex of IgG and active TGFβ1 is greatly accelerated in autoimmunity, and that this complex is extremely potent for suppression of PMN function and host defense against bacterial infection. (*J. Clin. Invest.* 1996. 98:2496–2506.) Key words: systemic lupus erythematosus • polymorphonuclear cells • B cells • phagocytosis • transforming growth factor

Introduction

Infection is a major cause of morbidity and mortality in SLE (1–3). In recent epidemiologic studies, infection was either the leading or one of the leading causes of death in SLE patients (4–6). Although immunosuppressive drug therapy contributes to the risk of infection, especially with opportunistic pathogens (4, 7), many host defense abnormalities which putatively predispose patients with SLE to infection have been described, including complement deficiencies, functional asplenia, reduced opsonic capacity, and defects in neutrophil (PMN) chemotaxis and phagocytosis (for review see reference 8). To determine the contribution of defects in PMN function to the predisposition to infection in SLE, previously we assessed PMN function and host defense against bacterial infection in a murine model of systemic autoimmunity, MRL/Mp-lpr/lpr (MRL/lpr)[1] mice (9, 10), in which host defense could be examined independently of therapeutic immunosuppression. Our studies indicated that MRL/lpr mice, as compared with congenic control mice, MRL/Mp-+/+ (MRL/n), (*a*) have an acquired defect in PMN phagocytic function and in PMN extravasation to an inflammatory site; (*b*) have significantly decreased survival in response to *Escherichia coli* and *S. aureus* infection at an age when their complement levels and kidney function are normal; (*c*) elaborate excessive levels of both the active and latent forms of the cytokine TGFβ in spleen cell cultures and at the site of bacterial infection; and (*d*) have enhanced resistance to a lethal bacterial challenge and normal PMN extravasation when treated with a monoclonal antibody (mAb 1D11) which neutralizes the activity of TGFβ (9, 10). These data suggest that the defects in PMN function and in host defense against bacterial infection in these mice are caused by elevated levels of active TGFβ circulating in the blood and at the site of infection. From these observations we hypothesized that elaboration of active TGFβ during the course of an autoimmune disease is an attempt to regulate autoimmunity and results in the inadvertent suppression of host defense against bacterial infection.

TGFβ plays contradictory roles in the pathogenesis of autoimmune diseases, exhibiting both immunosuppressive and proinflammatory properties (11). These divergent roles are clearly exemplified in studies of mice made genetically deficient in the expression of TGFβ1 and in transgenic mice which overproduce active TGFβ1 (12–14). TGFβ1 knockout mice develop a similar pattern of autoantibodies as MRL/lpr mice and have IgG deposits in their renal glomeruli (12, 13). In contrast, TGFβ1 transgenic mice have fibrotic lesions in many organs and develop glomerulonephritis resembling immune complex–mediated glomerular injury (14). These studies and others clearly define an important role for TGFβ1 in suppressing autoreactivity while at the same time promoting tissue injury as a consequence of immune complex deposition (12–17). This dichotomy in function may derive from either the local or systemic production of active TGFβ (15, 18, 19), the cellular source of the TGFβ (13, 20), or the molecular form of the TGFβ involved (21, 22). In this last regard, TGFβ has been re-

Address correspondence to Hattie D. Gresham, Ph.D., Rm. 379, BMSB, Department of Microbiology and Immunology, School of Medicine, University of New Mexico, Albuquerque, NM 87131-5276. Phone: 505-277-5764; FAX: 505-277-6029.

Received for publication 15 August 1996 and accepted in revised form 2 October 1996.

1. *Abbreviations used in this paper:* BML, bone marrow leukocytes; E, erythrocytes; MRL/lpr, MRL/Mp-lpr/lpr; MRL/n, MRL/Mp-+/+; PI, phagocytic index; SA1, *Staphylococcus aureus* SA1;TBS, Trizma buffer solution.

ported recently to bind IgG antibodies and in this form has suppressive activity in vitro for cytolytic T lymphocyte function (21) and B lymphocyte proliferation (22). Moreover, TGFβ has been found in association with IgG in young MRL/lpr mice (23). However, the role of IgG-bound TGFβ in suppressing PMN function and host defense against bacterial infection has not been examined. Therefore, the goals of this work are to identify the molecular form and the cellular source of the active TGFβ isoform(s) which mediate suppression of PMN function and host defense against bacterial infection in systemic autoimmunity.

Our present data indicate that TGFβ1 is in the active form inside B cells and plasma cells, that it is found in the active form complexed to IgG in the plasma and in supernatants of purified B cells and plasma cells, that the complex of TGFβ1 bound to IgG is many times more potent than recombinant active TGFβ1 for suppression of PMN function in vitro and host defense against *S. aureus* SA1 infection in vivo, and that this complex can be found in the plasma of some patients with SLE. To our knowledge, this represents the first demonstration of active, mature TGFβ1 inside a cell.

Methods

Mice. MRL/Mp-lpr/lpr (MRL/lpr), MRL/Mp-+/+ (MRL/n), and BALB/c ByJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). MRL/n and MRL/lpr mice were bred and maintained in microisolator housing units (Lab Products, Inc., Maywood, NJ) under specific pathogen-free conditions as described previously (10). To study an additional murine model of systemic autoimmunity, we induced autoimmunity in 8-wk-old female BALB/c mice by injection of 0.5 ml of sterile, endotoxin-free pristane (Sigma, St. Louis, MO) and control mice were injected with sterile saline (24). After 8–13 wk, systemic autoimmunity was evident (24) and the mice were studied for PMN function and host defense status. Heparinized blood was collected from the superior vena cava of mice anesthetized with 100 μl of a solution containing 5 mg/ml ketamine HCl (Fort Dodge Labs, Fort Dodge, IA) and 1 mg/ml rompin (Miles, Shawnee, KS) administered intraperitoneally (i.p.). Platelet-depleted plasma was prepared by centrifugation for 10 min at 2,000 rpm at room temperature to remove erythrocytes followed by a second centrifugation at 10,000 *g* for 10 min at room temperature to remove platelets. Femurs were removed aseptically and flushed with HBSS/0.1% FBS (Gibco Laboratories, Grand Island, NY) to obtain bone marrow leukocytes (BML). Neutrophils (PMNs) were isolated from BML using NIM2 isolation media (Cardinal Associates, Inc., Santa Fe, NM) following the manufacturer's instructions. The cells were 75–80% PMN as determined by staining with 0.1% cresyl violet.

SLE patients and normal volunteers. Heparinized plasma and PMN, purified as described (9), were obtained from a total of 18 randomly selected patients meeting the 1982 ACR criteria for the classification of SLE and 9 age- and gender-matched normal volunteers. All SLE patients were followed at the University of Missouri-Columbia Hospital and Clinics, all studies were approved by the UMC Institutional Review Board, and patient consent was obtained.

Bacterial challenge. Bacterial challenge of animals was performed as described previously (10). The pathogen used in these studies, *S. aureus* strain SA1, an encapsulated and TSST-1 positive clinical isolate, was provided by Dr. J. Lee (Harvard University, Cambridge, MA). To maintain virulence, bacteria were animal passaged immediately before injection into experimental animals. SA1 were suspended in pyrogen-free, sterile normal saline and adjusted to the desired inoculum spectrophotometrically at 530 nm before injection. The inoculum used for bacterial challenge was confirmed by serial dilution in sterile normal saline and each dilution was counted by a spread-plate method onto blood agar media (Becton Dickinson, Cockeysville, MD). Survival in response to i.p. SA1 challenge and the percentage of PMN, number of PMN/ml, and the CFU/ml of a 5-ml peritoneal lavage obtained 24 h after i.p. challenge were performed as described previously (10).

Purification of B cell–plasma cell preparations. Spleens were removed aseptically from age- and sex-matched MRL/n, MRL/lpr, saline-injected BALB/c, and pristane-injected BALB/c mice and dispersed in 5 ml HBSS buffer (HBSS containing 10% FBS, and 10 mM Hepes) followed by RBC lysis with a 10× volume of 17 mM ammonium chloride, pH 7.2 (Sigma) for 10 min at room temperature. Splenocytes were washed with HBSS buffer containing 10 mM EDTA to remove platelets as described previously (10). To enrich for B cells and plasma cells, splenocyte suspensions were depleted of T cells, monocytes, macrophages, and granulocytes by negative selection. Briefly, splenocytes (1×10^8) were incubated for 30 min on ice with a 10-ml mixture of the following hybridoma culture supernatants (2 ml of each): 30H12 (rat IgG2b anti–mouse Thy1.2), GK1.5 (rat IgG2b anti–mouse CD4), 53.6 (rat IgG2b anti–mouse CD8), M1/70 (rat IgG2b anti–mouse CD11b), and RB6 (rat IgG2b anti–granulocyte-1). Antibody-coated cells were pelleted at 1,200 rpm for 10 min at 4°C, washed with cold HBSS buffer, and incubated with 4 ml goat anti–rat IgG magnetic particles (Perspective Diagnostics, Cambridge, MA) in cold HBSS buffer for 20 min on ice. All cells bound by magnetic particles were removed by duplicate rounds of magnetic depletion for 15 min at room temperature. The enriched B cell–plasma cells preparations were assessed by flow cytometry for expression of surface IgG and were > 86% positive for surface IgG and did not express CD4, CD8, CD11b, or Gr-1. Cytospin preparations were made from 100 μl of the enriched B cells–plasma cells (2×10^6 cells/ml) and stained with Wright's stain to assess the percentage of plasma cells by light microscopy which ranged from 18 to 35% (not all plasma cells are surface IgG positive). In addition, a rare megakaryocyte was observed on the Wright's stained preparations. To assess secreted IgG-bound TGFβ1, purified B cells–plasma cells (4×10^6 cells/ml) were cultured in DMEM media supplemented with either 5% FBS or 1% Nutridoma (Boehringer-Mannheim, Indianapolis, IN) in sterile 4-well flat-bottom polystyrene culture plates (Becton Dickinson Labware, Lincoln Park, NJ). Cells were cultured either in media alone or in the presence of 10 ng/ml murine IL-4 (R & D Systems, Minneapolis, MN) and 1 μg/ml lipopolysaccharide (LPS from *E. coli* O111:B4, Sigma) to stimulate Ig secretion. After 24 h, supernatants were collected and IgG was isolated, quantitated, and both total and active TGFβ1 levels were determined as indicated.

IgG isolation. IgG was isolated from murine and human plasma, 24-h peritoneal lavage samples after i.p. challenge with SA1, and culture supernatants from purified B cells and plasma cells using the mAb Trap GII antibody purification kit (Pharmacia Biotech, Alameda, CA) according to the manufacturer's instructions. Column eluates containing IgG were lyophilized and reconstituted to the original volume. Concentrations of IgG present in samples were determined by radial immunodiffusion as compared with standards using a mouse IgG NL RID kit (Binding Site, Inc., San Diego, CA).

Preparation of antibody. Murine IgG1 monoclonal anti-TGFβ (clone 1D11) which recognizes the active forms of TGFβ1, TGFβ2, and TGFβ3, prepared as described (25), or murine IgG1 control, KG7, were provided by Celtrix Pharmaceuticals (Santa Clara, CA). Before use or injection, all antibodies were subjected to buffer exchange to ensure the absence of endotoxin as described previously (10). All antibody concentrations were adjusted to 1 mg/ml.

TGFβ assay. To accurately measure TGFβ, all tubes, plates, etc. were coated with 0.2% BSA to reduce nonspecific binding of TGFβ to plastic. Total TGFβ1 levels in plasma and peritoneal lavage samples were analyzed by a TGFβ1 ELISA kit (Promega, Madison, WI) and total TGFβ2 levels in plasma and lavage samples were analyzed by a TGFβ2 Quantikine ELISA kit (R & D Systems) following acid-ethanol extraction as described by Danielpour (26). Briefly, to 1 ml of lavage or plasma 4.0 ml of cold acid-ethanol solution containing 93%

ethanol, 0.24 M HCl, 85 μg/ml phenylmethylsulfonyl fluoride, and 5 μg/ml pepstatin A (Sigma) was added and extraction was performed overnight with gentle rocking at 4°C. The samples were clarified by centrifugation at 10,000 *g* for 10 min and the supernatants were dialyzed against 4 mM HCl overnight at 4°C using Slide-A-Lyzer 10K dialysis cassettes (Pierce, Rockford, IL). Samples were clarified by centrifugation, as above, and resulting supernatants were flash frozen in liquid N_2 and lyophilized using a Virtis Freezemobile 25 lyophilization unit (Virtis Co., Inc., Gardiner, NY). Lyophilized samples were reconstituted with 1 ml sterile water and total TGFβ1 and TGFβ2 levels were analyzed by ELISA following the manufacturer's instructions. To measure the levels of TGFβ3 in plasma, the CCL-64 mink lung cells (American Type Culture Collection, Rockville, MD) bioassay was used as described previously (10). Briefly, acid-ethanol extracted plasma (0.5 ml) was incubated with 50 μg of either polyclonal neutralizing anti-TGFβ3 (R & D Systems), anti-TGFβ1, β2, β3 (1D11) monoclonal antibody as a positive control, or gIgG or KG7 as a negative control for 1 h at 0°C before analysis in the bioassay. The bioactivity in the presence of anti-TGFβ3 was compared with the bioactivity in the presence of control antibody to assess the presence of TGFβ3 biological activity. The TGFβ1 Predicta ELISA kit (Genzyme Corp., Cambridge, MA) was used to measure the levels of both active and total TGFβ1 in culture supernatants and in IgG purified from either plasma or B-plasma cell culture supernatants as described by Schultz-Cherry et al. (27). The TGFβ1 Predicta ELISA uses mAb 1D11 and therefore is selective only for active TGFβ1. To measure active TGFβ1, 200 μl of culture supernatant or purified IgG in PBS was diluted 1:2 and directly analyzed following the manufacturer's instructions. For measurement of total (active + latent) TGFβ1, 200 μl of sample was diluted 1:2 and acid activated with 20 μl of 1 N HCl for 1 h on ice and neutralized with 20 μl of 1 N NaOH before analysis as directed in the instructions. An additional standard of 0.04 ng/ml was used to construct the standard curve.

Opsonization of sheep erythrocytes. Sheep erythrocytes (E) (Whittaker M.A. Bioproducts, Walkersville, MD) were opsonized with murine monoclonal IgG2b anti–sheep E (Accurate Chemical and Scientific Co., Westbury, NY) for murine PMN phagocytosis assays or with rabbit IgG anti–sheep E (Diamedix, Miami, FL) for human PMN phagocytosis assays as described previously (9). Opsonized E (EIgG2b or EIgG) were suspended at 5×10^8/ml in dextrose-gelatin veronal-buffered saline.

Phagocytosis assays. PMN phagocytosis was assessed by a fluid-phase assay as described previously (9). Human peripheral blood PMN or murine BML-derived PMN (2×10^5) were incubated with 15 μl of EIgG2b or EIgG in the presence of buffer alone (HBSS containing 1.0% HSA, 10 mM Hepes, 4.2 mM sodium bicarbonate, 1.0 mM Ca^{2+}, and 1.0 mM Mg^{2+}) or buffer containing either 50 μM FMLP (Sigma) or the indicated concentration of PDBu (Sigma) in a final volume of 115 μl. In addition, where indicated, PMN were incubated with IgG-bound TGFβ1 purified from either B-plasma cell supernatants or plasma in the presence or absence of 1D11 or KG7 antibody followed by repeated washes in buffer alone before assay. To allow the target particles to interact optimally with the PMN, the reaction mixtures were centrifuged at 200 *g* for 1 min and gently resuspended before incubation at 37°C. After 30 min, uningested E were lysed by the addition of 1 ml of cold 0.83% ammonium chloride. Phagocytosis was assessed by light microscopy and quantitated as a phagocytic index (PI), the number of EIgG2b or EIgG ingested/100 PMN.

Immunohistochemistry. We followed the immunohistochemistry protocol of Pelton et al. (28) with minor modifications. To assess intracellular active TGFβ, splenic B-plasma cell cytospin preparations were fixed in 95% ethanol with 5% acetic acid. Methanol fixation was also used with equivalent results. Slides were submerged in TBS (Trizma buffer solution) containing 0.1% (vol/vol) Triton X-100 (Sigma) at room temperature for 15 min followed by TBS for 5 min, methanol for 2 min, and 0.6% (vol/vol) hydrogen peroxide in methanol for 30 min. Slides were subsequently washed at room temperature in methanol for 2 min, TBS for 5 min, and three times in TBS containing 0.1% (wt/vol) BSA for 3 min. After treatment with hyaluronidase (1 mg/ml in 100 mM sodium acetate, 0.85% [wt/vol] NaCl) for 30 min at 37°C and three washes in TBS/0.1% BSA, B-plasma cell slides were treated with an Avidin-Biotin block (Vectastain) for 15 min at room temperature, rinsed in TBS, and blocked with 1% goat serum in TBS containing 0.5% BSA for 30 min at room temperature. B-plasma cell slides were incubated with 100 μg/ml biotinylated anti-TGFβ1 (1D11) or KG7 (control IgG1) in TBS containing 1% goat serum and 0.1% BSA overnight at 4°C in a humidity chamber. 1D11 and KG7 were biotinylated using a biotin/avidin reagent (Zymed, Laboratories, Inc., South San Francisco, CA) according to the manufacturer's instructions. Cytospin preparations were washed three times in TBS with 0.1% BSA, slides were exposed to ABC complex, followed by 0.05% DAB and 0.1% hydrogen peroxide. Cytospins were counterstained with Gill's No. 2 hematoxylin (Fisher Scientific, Pittsburgh, PA) and examined under light microscopy.

Statistical analysis. Data are presented as the mean±SEM. Statistical analyses were determined by the Mann-Whitney U test for nonparametrics or the Fisher's exact test using Statview 4.01 by Abacas Concepts, Inc. (Berkeley, CA) for Macintosh.

Results

TGFβ1 is the predominant isoform increased in the plasma and at the site of S. aureus SA1 infection in MRL/lpr mice. We demonstrated previously that increased susceptibility to bacterial infection in MRL/lpr mice was due to the spontaneous elaboration of TGFβ (10). To assay which isoform was responsible, the total level (latent + active) of each isoform in plasma and of a lavage from the site of infection was assessed. Pooled heparinized plasma and peritoneal lavages obtained 24 h after i.p. infection with SA1 were acid-ethanol extracted to remove proteins which bind to TGFβ and interfere with its detection (26). As shown in Fig. 1, MRL/lpr mice have significantly higher levels of total TGFβ1 both in the plasma and at the site of infection (Fig. 1 *A*). TGFβ2 levels were considerably lower and ranged from 10 to 75 pg/ml in both plasma and at the site of infection (data not shown). No detectable levels of TGFβ3 were found in either plasma or lavage samples from either MRL/n or MRL/lpr mice (data not shown). These data demonstrate that TGFβ1 is the predominant isoform overexpressed in both the plasma and at the site of infection in MRL/lpr mice.

IgG-bound TGFβ1 is significantly elevated in plasma and at the site of S. aureus SA1 infection in MRL/lpr mice. Recently, TGFβ has been reported to bind IgG in both autoimmune and normal murine models (21, 23). However, the TGFβ isoform associated with IgG and whether IgG-bound TGFβ1 is also elevated at sites of infection have not been determined. To determine if there is a role for IgG-bound TGFβ1 in the increased susceptibility of these mice to infection, we measured total IgG-bound TGFβ1 in pooled plasma and in 24 h SA1 peritoneal lavage (Fig. 1 *B*). MRL/lpr mice have significantly elevated levels of IgG-bound TGFβ1 in plasma as compared with MRL/n mice. Moreover, the amount of TGFβ1 associated with IgG comprised ~ 75% of the total TGFβ1 circulating in the plasma of MRL/lpr mice. In addition, MRL/lpr mice had significantly more IgG-bound TGFβ1 at the site of infection as compared with MRL/lpr mice. Neither TGFβ2 nor TGFβ3 was detected bound to IgG in either plasma or lavages from MRL/n or MRL/lpr mice (data not shown). Thus, MRL/lpr mice have significantly elevated levels of IgG-bound TGFβ1 in both plasma and at the site of SA1 infection as com-

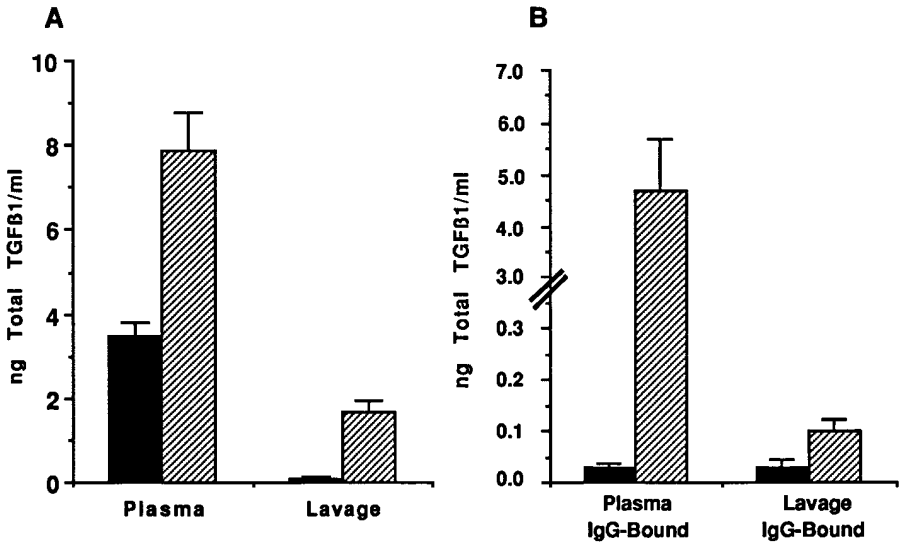


Figure 1. Comparison of MRL/n (*blacks bars*) and MRL/lpr (*striped bars*) mice for total TGFβ1 levels (*A*) and IgG-bound TGFβ1 levels (*B*) in plasma and at the site of infection. (*A*) Pooled heparinized plasma from 10–12-wk-old female mice and peritoneal lavages obtained from 14-wk-old female mice 24 h after i.p. infection with 3×10^5 CFU of SA1 were acid-ethanol extracted and TGFβ1 was measured by ELISA. Levels of total TGFβ1 in plasma were significantly different between MRL/n ($n = 9$ pools, 2 mice/pool) and MRL/lpr mice ($n = 10$ pools, 2 mice/pool), $P = 0.02$, Mann-Whitney U test. Levels of total TGFβ1 in the peritoneal lavage were significantly different between MRL/n ($n = 5$) and MRL/lpr mice ($n = 6$), $P = 0.006$, Mann-Whitney U test. (*B*) IgG purified from plasma and peritoneal lavages was acid-ethanol extracted and IgG-bound TGFβ1 was measured by ELISA. Plasma levels of total IgG-bound TGFβ1 from 10–16-wk-old male MRL/n ($n = 4$ pools, 3 mice/pool) and MRL/lpr mice ($n = 4$ pools, 3 mice/pool) were significantly different, $P = 0.02$, Mann-Whitney U test. Levels of total IgG-bound TGFβ1 in peritoneal lavages from 14-wk-old female MRL/n ($n = 4$) and MRL/lpr mice ($n = 4$) after i.p. infection with 2×10^5 CFU SA1 were significantly different, $P = 0.021$, Mann-Whitney U test. MRL/lpr mice have significantly higher levels of TGFβ1 in both plasma and at the site of infection. Moreover, the majority of the TGFβ1 in MRL/lpr plasma is bound to IgG.

pared with MRL/n mice. Moreover, the predominant form of TGFβ1 circulating in the plasma of autoimmune MRL/lpr mice is IgG-bound.

IgG-bound TGFβ1 is secreted in the active form from purified B cells and plasma cells from MRL/lpr mice and this complex is extremely potent for suppression of host defense against infection in vivo and for suppression of PMN function in vitro. Our previous data indicated that unstimulated splenocytes from MRL/lpr mice produced more active and total TGFβ in culture than did MRL/n splenocytes (10). While this pointed to hematopoietic cells as the source of the active TGFβ involved in suppression of host defense, the cellular source was not identified. Because active recombinant TGFβ1 has been shown to bind to purified IgG (22), IgG-bound TGFβ1 could arise in the periphery by release of active TGFβ1 from multiple sources. Alternatively, the active TGFβ1 bound to IgG could derive from the B cells and plasma cells which secreted the IgG, as postulated by others (23). To test this we examined the ability of active recombinant TGFβ1 to bind to IgG in MRL/n plasma and to purified murine IgG. We added 20 ng of active recombinant TGFβ1/ml of MRL/n plasma to 5 mg/ml of purified murine IgG in buffer and incubated the mixture for 15 min at 37°C. We then isolated the IgG from the mixture and measured the amount of TGFβ1 associated with the IgG. Unlike the results obtained by others (22), we found that none of the added recombinant TGFβ1 became associated with the IgG either in plasma or in buffer (data not shown), thus it is unlikely that the complex arises in the periphery. Therefore, we tested the ability of purified B cells and plasma cells from spleens of MRL/n and MRL/lpr mice to secrete IgG-bound TGFβ1. Purified B cells and plasma cells stimulated with IL-4 and LPS to induce Ig secretion secreted IgG-bound TGFβ1, and both the amount of IgG and the amount of TGFβ1 in the complex were increased in MRL/lpr mice (Table I). In addition, TGFβ1 was bound to IgG released from MRL/lpr B cells and plasma cells in the absence of additional exogenous stimulation (Table I). Equivalent results were obtained in the presence of either 5% FBS or 1% Nutridoma, ruling out the possibility that the TGFβ1 in the complex was coming from FBS (experiment 1 versus experiment 2). In addition, neither TGFβ2 nor TGFβ3 was detected bound to IgG secreted from B-plasma cells. These data indicate that supernatants from purified B cells and plasma cells contain TGFβ1 which is bound to IgG.

In our previous work, mAb 1D11, which recognizes an epitope on active TGFβ and neutralizes its biologic activity (25), augmented host defense against infection (10). Because our present data suggested that the majority of the TGFβ1 elevated in these mice is bound to IgG, this suggested that the TGFβ1 bound to IgG is in the active and not in the latent form. To assess this, we purified the IgG from the purified B cell and plasma cell culture supernatants and measured the amount of active TGFβ1 bound, without acid ethanol extraction, using an ELISA based on the availability of the 1D11 epitope. As shown in Table I, the majority of TGFβ1 bound to IgG in these supernatants was already in the active form and acid activation to detect total levels only modestly increased this amount. Again, this process was greatly accelerated in MRL/lpr mice. These data indicate that IgG-bound TGFβ1 secreted by purified B cells and plasma cells in culture expresses the 1D11 epitope even though it is complexed to IgG.

A trivial explanation for these data could be that the buffer used for eluting the IgG from the column is capable of activating latent TGFβ1. To test for this, we incubated a platelet lysate containing latent TGFβ1 with the elution buffer and measured the amount of TGFβ1 detectable based on the availability of the 1D11 epitope. No active TGFβ1 was detected. An additional control included chromatographing the culture supernatants over gelatin-Sepharose to assess whether TGFβ1 was binding nonspecifically to the Sepharose column. An elu-

Table I. Comparison of Purified B Cell–Plasma Cell Culture Supernatants for IgG-bound TGFβ1

		IgG-bound	
	IgG*	Active TGFβ1‡	Total TGFβ1‡
	mg/ml	*ng/ml*	*ng/ml*
Experiment 1			
MRL/n			
Media control	0.0	< 0.04	< 0.04
IL-4 + LPS	0.8	< 0.04	< 0.04
MRL/lpr			
Media control	0.9	1.23	1.46
IL-4 + LPS	2.0	4.61	5.33
Experiment 2			
MRL/n			
Media control	0.0	< 0.04	< 0.04
IL-4 + LPS	0.6	0.80	1.00
MRL/lpr			
Media control	0.8	1.17	1.31
IL-4 + LPS	1.8	3.61	4.52

Comparison of active and total IgG-bound TGFβ1 levels in 24 h culture supernatants from unstimulated (media alone) and stimulated (10 ng/ml IL-4 + 1 μg/ml LPS) purified B cells and plasma cells obtained from 16-wk-old female MRL/n ($n = 2$) and MRL/lpr ($n = 2$) mice. Cells were grown in DMEM/5% FBS/1% glutamine/20 mM Hepes/50 μg/ml gentamycin/50 μm 2-mercaptoethanol (*Experiment 1*) or DMEM/1% Nutridoma/1% glutamine/20 mMN Hepes/50 μg/ml gentamycin (*Experiment 2*). MRL/n purified B cells and plasma cell cultures contained 18% plasma cells in experiments 1 and 2 and MRL/lpr B-plasma cell cultures contained 35% plasma cells in experiment 1 and 27% plasma cells in experiment 2, respectively. After 24 h, MRL/lpr purified B cell and plasma cell cultures contained higher levels of IgG-bound TGFβ1. IgG-bound TGFβ1 is predominantly in the active form. *Culture supernatants were passed over protein G–Sepharose columns and the IgG concentration of the eluate was determined by radial immunodiffusion for murine IgG. ‡Assessment of both active and total levels of IgG-bound TGFβ1 was performed by ELISA using the TGFβ1 Predicta kit as described in Methods.

ate of the gelatin Sepharose column did not contain active TGFβ1 by ELISA.

These data suggest that the TGFβ1 bound to IgG is in the biologically active form at the time it is secreted from the B cells and plasma cells. To test the biologic activity of the purified complex, we assessed the ability of the IgG-bound TGFβ1 purified from MRL/n and MRL/lpr B cells and plasma cells to increase the susceptibility of MRL/n mice to infection with *S. aureus* SA1. To our surprise, injection of only 100 μg of IgG purified from MRL/lpr B-plasma cells containing 200 pg of active TGFβ1 at the time of bacterial challenge significantly reduced the survival of MRL/n mice to 0% (Fig. 2 *A*). In contrast, 100 μg of MRL/n IgG, containing < 5 pg of active TGFβ1, did not affect survival of MRL/n mice (Fig. 2 *B*). Because 100 ng of active recombinant TGFβ1 is required to significantly affect host defense against SA1 infection in MRL/n mice (10), IgG-bound TGFβ1 is 500 times more potent for suppression of host defense in vivo than is recombinant active TGFβ1. These data are consistent with our inability to demonstrate binding of exogenously added recombinant TGFβ1 to IgG in MRL/n plasma and may explain this difference in potency (see above). To verify that this complex was sufficient to suppress PMN function, we investigated the effect of the IgG-bound TGFβ1 complex purified as above on the ability of PMN to increase ingestion of IgG-opsonized targets in response to stimulation with the chemotactic peptide, FMLP, as described previously (9). Incubation of bone marrow–derived PMN from MRL/n mice with 20 μg of IgG purified from MRL/lpr cell supernatants and containing 40 pg of active TGFβ1 suppressed stimulated phagocytosis by MRL/n PMN to a level comparable with MRL/lpr PMN (Fig. 2 *B*). Moreover, the suppression mediated by MRL/lpr IgG could be overcome by pretreatment with mAb 1D11 but not with KG7 control antibody. Thus, IgG-bound TGFβ1 from MRL/lpr mice, in the absence of any exogenous activation step, is fully sufficient to suppress stimulated phagocytosis by normal PMN. These data indicate that the IgG-bound TGFβ1 complex produced by purified B cells and plasma cells of autoimmune mice is secreted in a biologically active form and is extremely potent for suppression of host defense in vivo and PMN function in vitro.

TGFβ1 is present in the active form inside B-plasma cells. TGFβ is secreted by cells primarily in a latent form and its conversion to the biologically active form occurs extracellularly (29). Since our present data indicated that the IgG-bound TGFβ1 was secreted in the active form, we hypothesized that TGFβ1 might exist in the active form inside B cells and plasma cells of these mice. To investigate this possibility, we performed immunohistochemical analysis for intracellular active TGFβ in purified B cells and plasma cells from MRL/n and MRL/lpr mice using mAb 1D11 to detect the active form of TGFβ (24). As shown in Fig. 3, B cells and plasma cells from MRL/n (Fig. 3, *C* and *E*) and MRL/lpr (Fig. 3, *D* and *F*) mice exhibit positive staining with 1D11 mAb in the cytoplasm and this positivity was most pronounced in plasma cells. MRL/n and MRL/lpr B-plasma cells treated with control KG7 antibody exhibited no positivity (Fig. 3, *A* and *B*, respectively). Moreover, plasma cells from MRL/lpr mice stained more intensely as compared with cells from MRL/n mice. The rare megakaryocyte present in these cell preparations was negative for 1D11 staining, indicating that 1D11 is not able to recognize latent intracellular TGFβ (Fig. 3 *F*, *open arrow*). When crude spleen cell suspensions (not depleted of any cell population) were examined for intracellular active TGFβ, neutrophils, macrophages, megakaryocytes, and the majority of lymphocytes were all negative for 1D11 positivity (data not shown). These data are consistent with the data in Table I and suggest that TGFβ1 is present in the active form inside plasma cells. In addition, the amount of active TGFβ1 inside B cells and plasma cells is vastly enhanced in autoimmune MRL/lpr mice. To our knowledge, this is the first demonstration of the exposure of the 1D11 epitope of mature TGFβ inside a cell.

IgG-bound TGFβ1 suppresses host defense and PMN function in other murine models of systemic autoimmunity and in patients with SLE. To verify that our results were not exclusive to the MRL/lpr animal model, we examined whether IgG-bound TGFβ1-mediated defects in PMN function and host defense against bacterial infection could be observed in other murine models of systemic autoimmunity and in patients with SLE. We chose to study pristane-induced systemic autoimmunity (24) because this model would allow us to examine host defense against bacterial infection under conditions where

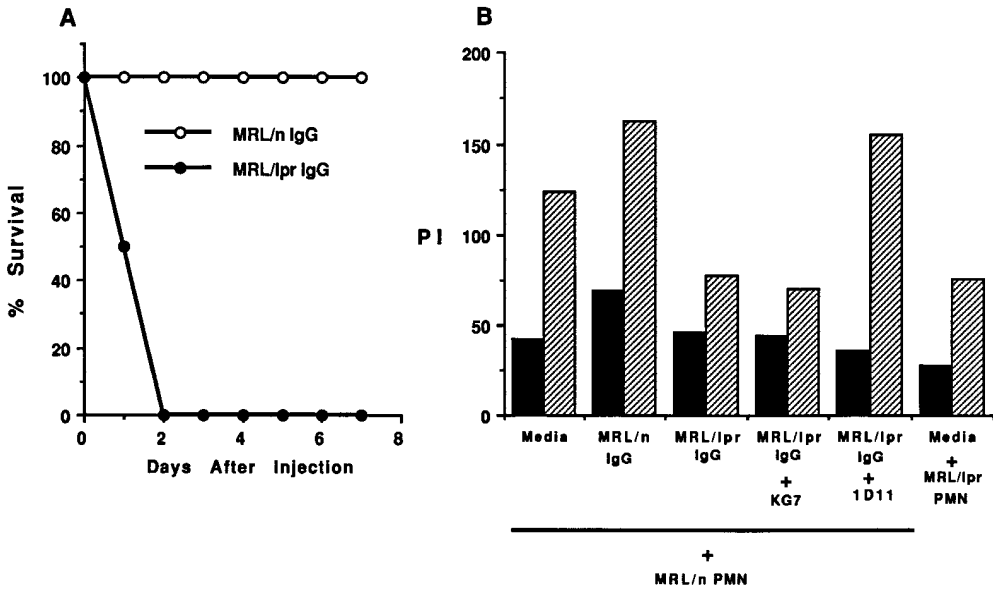
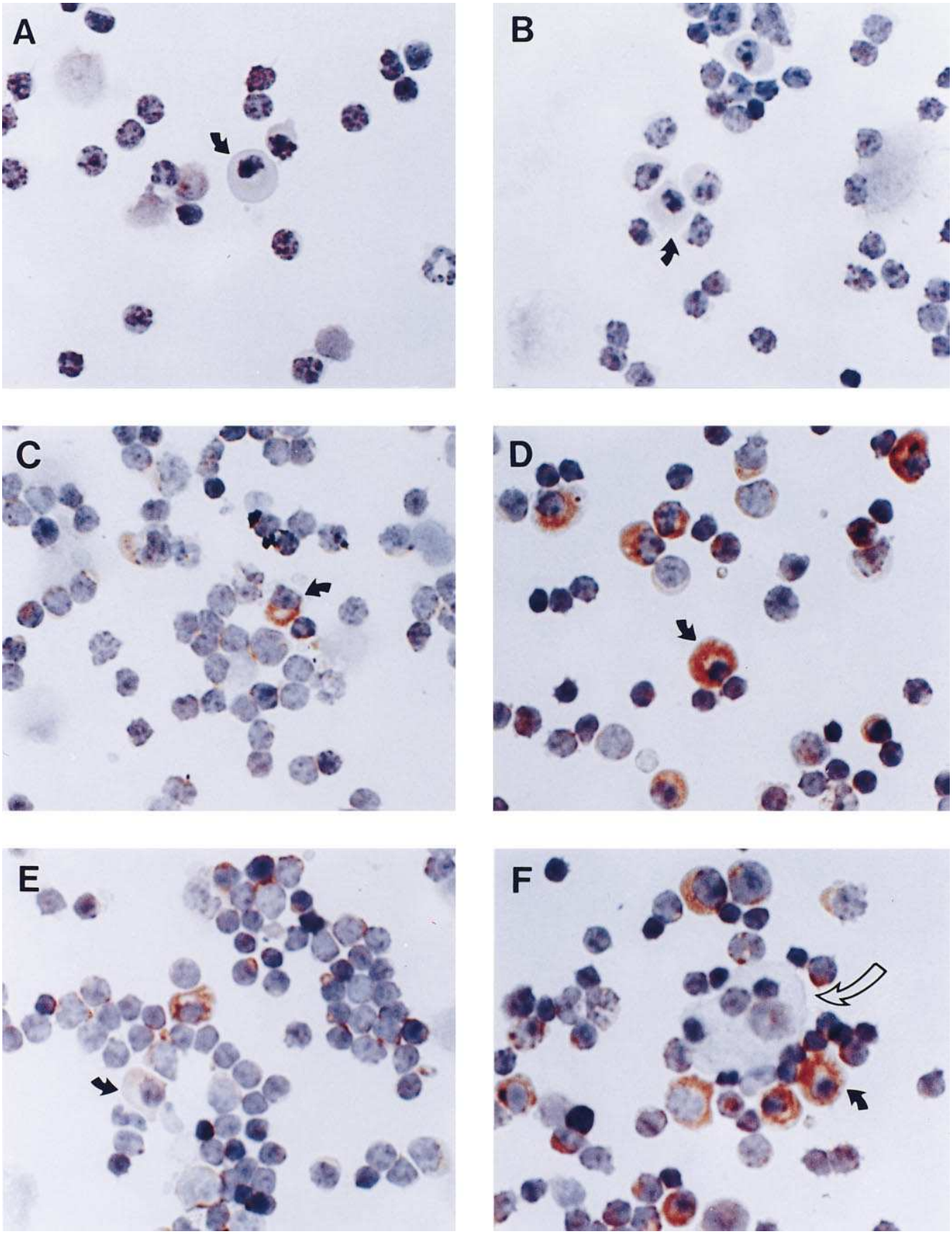


Figure 2. Effect of IgG-bound TGFβ1 from MRL/n and MRL/lpr mice on (*A*) the survival of MRL/n mice in response to SA1 infection and (*B*) the phagocytic function of MRL/n PMN. (*A*) IgG was purified from IL-4 and LPS-stimulated B-plasma cell culture supernatants (see Table I) and 100 μg purified from MRL/n mice, containing < 5 pg of active TGFβ1, and from MRL/lpr mice, containing 200 pg active TGFβ1, was injected intravenously at the time of i.p. infection with 1.5×10^5 CFU of SA1. The percentage of mice surviving was assessed daily for 7 d. MRL/n mice injected with IgG-bound TGFβ1 purified from MRL/lpr mice (n = 6) had significantly decreased survival in response to SA1 infection as compared with mice injected with MRL/n IgG-bound TGFβ1 (n = 5), P = 0.002, Fisher's exact test. (*B*) Bone marrow–derived PMN (4×10^6) from 14-wk-old female MRL/n were incubated for 1 h at 37°C with 20 μg of IgG-bound TGFβ1 purified as above from either MRL/n (< 1 pg active TGFβ1) or MRL/lpr mice (40 pg active TGFβ1) as follows: media control; MRL/n IgG-TGFβ1; MRL/lpr IgG-TGFβ1; MRL/lpr IgG-TGFβ1 preincubated with 10 μg of isotype control antibody KG7 for 30 min at 0°C; and MRL/lpr IgG-TGFβ1 preincubated with 10 μg of anti-TGFβ 1D11 monoclonal antibody for 30 min at 0°C. PMN from age- and gender-matched MRL/lpr mice were examined as an additional control. Treated PMN (2×10^5) were incubated with EIgG2b in the presence of buffer alone (*black bars*) or 50 μM FMLP (*striped bars*). After 30 min at 37°C, uningested E were lysed and phagocytosis was assessed by light microscopy. PI is the amount of EIgG2b ingested by 100 PMN. IgG-bound TGFβ1 purified from B-plasma cells from MRL/lpr mice is extremely potent in suppressing host defense against infection and in suppressing PMN phagocytic function.

gene expression regulating host defense would be equivalent. We injected BALB/c mice with 0.5 ml of either sterile saline or pristane. After 9–13 wk, we examined the levels of IgG-bound TGFβ1 in the plasma and in culture supernatants from B cells and plasma cells, the phagocytic function of bone marrow PMN, and host defense against SA1 infection. In all experiments, pristane-treated BALB/c mice were equivalent to MRL/lpr mice and saline-treated BALB/c mice were equivalent to MRL/n mice. Pristane-treated mice had 5.18 ng of IgG-bound active TGFβ1/ml of plasma whereas saline-treated mice had levels below the detection of the assay (< 0.04 ng/ml). Moreover, pristane-treated mice had 2.22 ng of IgG-bound active TGFβ1/ml of a B cell and plasma cell culture supernatant containing 1.8 mg of IgG/ml whereas saline-treated mice had < 0.04 ng/ml of supernatant containing 0.8 mg/ml of IgG. Moreover, B-plasma cell cytospin preparations from pristane-treated mice exhibited more intense staining for intracellular active TGFβ than did saline-treated mice. Moreover, PMN from pristane-treated mice failed to augment ingestion in response to FMLP stimulation as compared with saline-treated mice (data not shown). These data demonstrate that induction of systemic autoimmunity results in the overproduction of IgG-bound active TGFβ1 and in an acquired defect in PMN function. To determine if the presence of this complex was sufficient to induce a defect in host defense, we challenged saline- and pristane-treated mice with SA1 i.p. After 24 h, we obtained a lavage of the peritoneum and assessed the percentage of PMN, number of PMN/ml of lavage, and the bacterial burden as CFU/ml of lavage. As shown in Fig. 4, pristane-treated mice had a significantly increased bacterial burden (Fig. 4 *C*) which resulted in a persistent influx of PMN as detected by both an increase in percentage of PMN (Fig. 4 *A*) and in increased numbers of PMN (Fig. 4 *B*). Moreover, treatment of the pristane-induced mice with mAb 1D11, as compared with the isotype control KG7, reversed their defects in host defense. These data are identical to those we published for MRL/lpr mice (10). These data indicate that pristane induction of systemic autoimmunity in BALB/c mice results in an acquired defect in host defense which is caused by IgG-bound TGFβ1 circulating in the vasculature.

To assess whether similar defects occur in patients with SLE as we described for MRL/lpr and pristane-treated BALB/c mice, we measured plasma TGFβ1 levels in 18 randomly selected patients meeting the ACR criteria for classification of SLE and 9 age- and gender-matched normal controls. As shown in Fig. 5 *A*, SLE patients had significantly higher plasma total TGFβ1 levels when compared with normals. We assessed PMN phagocytic function over time in two of the patients with the highest levels of TGFβ1 (> 13 ng/ml). As shown in Fig. 5 *B*, PMN from SLE patient 1 with 13.4 ng/ml of TGFβ1 in her plasma failed to enhance phagocytosis of IgG opsonized erythrocytes when stimulated with increasing concentrations of PDBu as compared with PMN from a normal control. When this same patient was examined 3 mo later, her plasma TGFβ1 level was normal (2.2 ng/ml) and her PMN phagocytic response to PDBu was equivalent to normal PMN (Fig. 5 *C*). Importantly, the first plasma TGFβ1 level was obtained while this patient was hospitalized with active lupus, and the subsequent level was obtained after treatment and clinical improvement, suggesting that these two parameters may vary


A
B
C
D
E
F

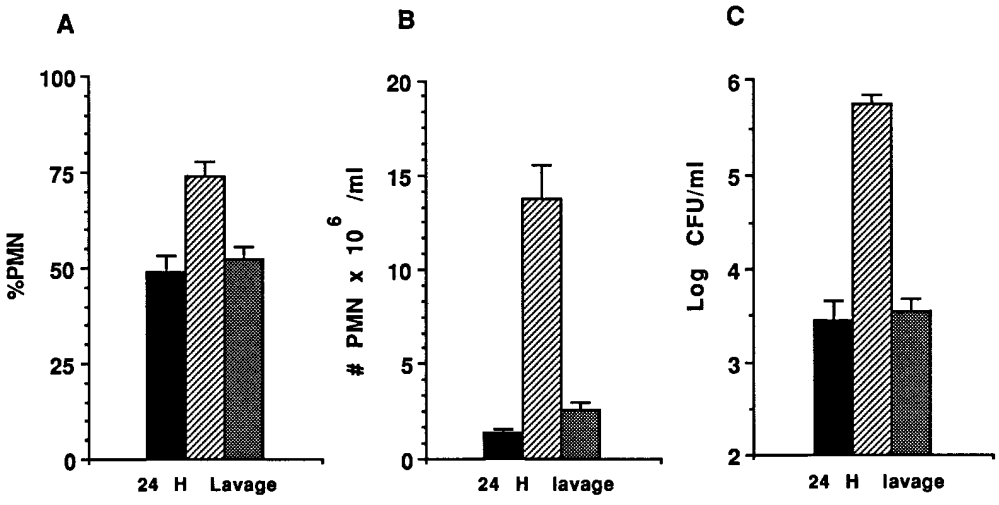


Figure 4. Comparison of saline-treated (*black bars*) and pristane-treated BALB/c mice injected i.v. with either 150 μg of mAb 1D11 (*shaded bars*) or isotype control mAb KG7 (*striped bars*) on the (*A*) percentage of PMN, (*B*) PMN number/ml, and (*C*) $\log_{10}$ CFU/ml of a lavage of the peritoneum 24 h after i.p. infection with 1×10^5 CFU of SA1. 8-wk-old female BALB/c mice were injected i.p. with either 0.5 ml of pristane or saline as a control 9 wk before the bacterial challenge. The percentage of PMN, PMN number/ml, and the CFU/ml were significantly different between mAb 1D11-treated ($n = 6$) and control KG7-treated ($n = 7$) mice that had been given pristane, $P = 0.0045$, 0.0027, and 0.0027, respectively, Mann-Whitney U test. The responses of the saline control mice ($n = 5$) were equivalent to those of the mAb 1D11–treated mice. Pristane induction of systemic autoimmunity in BALB/c mice increases their susceptibility to SA1 infection and mAb 1D11 treatment reverses their increased susceptibility.

with disease activity. Qualitatively similar data were obtained with SLE patient 2 demonstrating abnormal PMN responses and elevated plasma TGFβ1 (13 ng/ml) on one day (6/21/95) and then normal values on subsequent study (11/7/95). Currently, we presume that the cycling of these two patients is due to variations in disease activity and autoantibody production due to treatment. However, further study will be required to determine a correlation between disease activity and plasma TGFβ1 levels.

To prove that these differences were due to the presence of IgG-bound TGFβ1 in the plasma, we purified the IgG from the plasma of SLE patient 2 from these 2 d and assessed the ability of the IgG-bound TGFβ1 to suppress PMN phagocytic function as performed in Fig. 2 *B*. As shown in Fig. 6, incubation of autologous PMN with the IgG-bound TGFβ1, purified from plasma containing 13 ng/ml of TGFβ1, suppressed their ability to enhance ingestion in response to the optimal concentration of PDBu. Moreover, preincubation of the complex with mAb 1D11, as compared with the isotype control KG7, reversed this suppression. In addition, IgG purified from plasma when her TGFβ1 level was 1.78 ng/ml had no effect on PMN phagocytic function. These data indicate that some patients with SLE have elevated levels of TGFβ1 in the plasma, that it is present as a complex bound to IgG, and that in this form it is fully sufficient to suppress PMN function.

Discussion

Our data indicate that B cells and plasma cells are the primary source of the active TGFβ1 in the circulation of autoimmune MRL/lpr and pristane-treated BALB/c mice and that it is IgG-bound active TGFβ1 which is responsible for suppressing host defense. TGFβ1 bound to IgG is many times (~ 500) more potent than active recombinant TGFβ1 for suppression of PMN phagocytic function in vitro and host defense against bacterial infection in vivo. Moreover, this complex can be detected in the plasma of some patients with SLE and when present is responsible for suppression of PMN phagocytic function. These data imply that under circumstances of polyclonal B cell activation or chronic antigenic stimulation, a complex of IgG and active TGFβ1 could arise and when present in the circulation could lead to defects in PMN function and in host defense against infections caused by common pathogens. Therefore, under these conditions defects in PMN function would be acquired and could result in significant morbidity and mortality due to infection. In fact, an increased risk of bacterial infection and defects in PMN function have been described not only in patients with SLE (8) but in patients with rheumatoid arthritis (30, 31), diabetes mellitus (32, 33), AIDS (34, 35), and in cancer patients undergoing IL-2 therapy (36, 37). Importantly, both patients with AIDS and those receiving IL-2 are known to have elevated plasma TGFβ levels (38–40). These data in combination with our studies in patients with SLE suggest that overproduction of IgG-bound active TGFβ1 could underlie the acquired defects in PMN function observed in many chronic human diseases. Demonstration of the presence of this complex in the plasma of these patients will be required to confirm this speculation.

The most novel observation of this study is the demonstration of active TGFβ1 inside B cells and plasma cells whereas

Figure 3. Immunohistochemical analysis of active TGFβ1 inside splenic B-plasma cell cytospin preparations from 16-wk-old female MRL/n ($n = 2$) (*A*, *C*, *E*) and MRL/lpr mice ($n = 2$) (*B*, *D*, *F*). Ethanol-fixed B-plasma cell cytospins were stained with biotinylated mAb KG7 (isotype control) (*A* and *B*) or mAb 1D11 (anti–active TGFβ) (*C–F*) and counterstained with hematoxylin. No positivity was evident for cells stained with control KG7 (*A* and *B*). mAb 1D11 positivity was most pronounced in plasma cells (brown staining) (*closed arrows*, *C– F*), and this staining was more intense in plasma cells from MRL/lpr mice (*D* and *F*) as compared with MRL/n mice (*C* and *E*). Megakaryocytes were negative for active TGFβ (*F*) (*open arrow*). Active TGFβ is present intracellularly in B-plasma cells at or near the time of its association with IgG and this process is greatly accelerated in MRL/lpr mice.

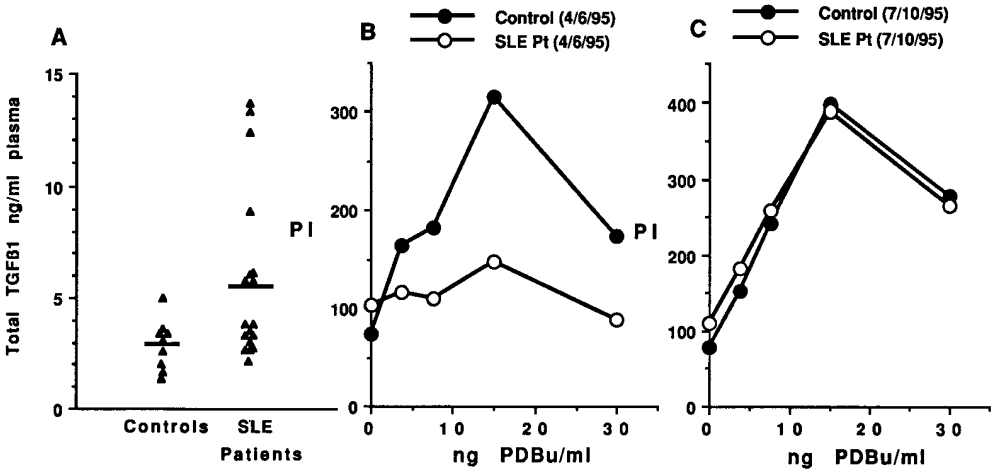


Figure 5. Assessment of total TGFβ1 levels in plasma of SLE patients as compared with healthy controls (*A*) and PMN phagocytic function of a patient with SLE when her plasma TGFβ1 level was 13.4 ng/ml (*B*) and when it was 2.2 ng/ml (*C*). (*A*) Platelet-depleted plasma was acid-ethanol extracted and TGFβ1 levels were measured by ELISA. Plasma TGFβ1 levels are significantly higher in patients with SLE ($n = 18$) than in normal controls ($n = 9$), $P = 0.038$, Mann-Whitney U test. (*B* and *C*) PMN were isolated from a normal control or SLE patient 1 on two separate days and their ability to augment ingestion of IgG-opsonized E in response to PDBu stimulation was assessed. PMN isolated from SLE patient 1 on a day when her plasma TGFβ1 level was 13.4 ng/ml (4/6/95) failed to augment ingestion over a wide dose range of PDBu. In contrast, when her plasma level returned to normal, 2.2 ng/ml (7/10/95), her PMN phagocytic function was equivalent to the normal control.

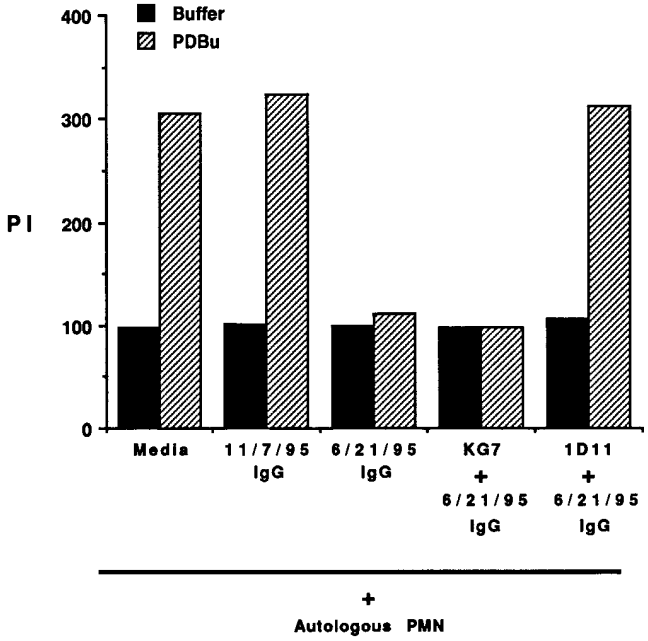


Figure 6. Effect of IgG-bound TGFβ1 purified from SLE patient 2 on the phagocytic function of autologous PMN. IgG-bound TGFβ1 was purified from platelet-depleted plasma from SLE patient 2 on a day when her plasma TGFβ1 level was 13 ng/ml (6/21/95) and when it was 1.78 ng/ml (11/7/95). Autologous PMN from 4/10/96 (4×10^6) were incubated for 1 h at 37°C with 150 μg of IgG-bound TGFβ1 purified from either 11/7/95 plasma (< 1 pg active TGFβ1) or 6/21/95 plasma (40 pg active TGFβ1) as follows: media control; 11/7/95 IgG-TGFβ1; 6/21/95 IgG-TGFβ1; 6/21/95 IgG-TGFβ1 preincubated with 10 μg of isotype control antibody KG7 for 30 min at 0°C; and 6/21/95 IgG-TGFβ1 preincubated with 10 μg of anti-TGFβ 1D11 monoclonal antibody for 30 min at 0°C. Treated PMN (2×10^5) were incubated with EIgG in the presence of buffer alone or 15 ng/ml of PDBu. After 30 min at 37°C, uningested E were lysed, and phagocytosis was assessed by light microscopy. PI is the amount of EIgG ingested by 100 PMN. IgG-bound TGFβ1 is present in SLE plasma and is fully sufficient to suppress PMN phagocytic function.

other spleen cell types were negative. These data suggest that the mechanism by which TGFβ1 is activated in association with IgG may be unique. TGFβ is known to be secreted by most cells in an inactive or latent form with the extracellular conversion to active TGFβ occurring after interaction with the cell surface serine protease, plasmin, and the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor (29). In contrast, our data demonstrate the presence of active TGFβ1 inside B cells and plasma cells. There are at least two possible mechanisms which could explain this result. First, colocalization of both TGFβ1 and IgG inside plasma cells could induce the conversion of TGFβ1 from the latent to the active form. In fact, IgG-induced activation could be very similar to the activation of TGFβ mediated by thrombospondin which is protease independent but occurs extracellularly (27). Like IgG, thrombospondin-bound TGFβ1 allows for expression of the mAb 1D11 epitope (27). IgG binding to the latent TGFβ complex could either induce a conformational change in the complex to expose residues required for TGFβ1 activity or induce the dissociation of active TGFβ1 from the latency-associated protein to expose the epitope recognized by mAb 1D11. Alternatively, TGFβ1 could be secreted in the latent form, activated, bound to secreted IgG, and then taken up as a complex via an endocytic mechanism by B cells and plasma cells. In this paradigm, any cell could be responsible for production of the TGFβ1. Currently, we do not favor this latter interpretation because we have been unable to detect binding of active recombinant TGFβ1 to murine IgG. We cannot currently explain why we have failed to see this when it has been observed by others (22). We favor the hypothesis that the TGFβ1 bound to IgG is made by antibody-secreting cells. To obtain evidence to support this, we have assessed the amount of active TGFβ1 bound to monoclonal antibodies purified from both tissue culture supernatant and from ascites. In fact, we have found substantial amounts of active TGFβ1 associated with monoclonal antibodies and in these circumstances the hybridoma cells themselves are both the source of the antibody and the source of the TGFβ1 (Gresham, H.D., unpub-

lished observations). Currently, experiments are in progress to definitively prove that TGFβ1 and IgG produced by the same cell become complexed intracellularly before their secretion.

Originally, we hypothesized that the excessive elaboration of TGFβ in autoimmunity may be a homeostatic mechanism for suppression of exaggerated and inappropriate immunostimulation (10). This hypothesis was in large part based on the considerable data indicating that TGFβ1 has potent immunosuppressive properties and because TGFβ1 knockout mice develop a systemic autoimmune disease (12, 13). However, our data indicate that TGFβ1 bound to IgG arises as a consequence of autoimmunity and that the autoimmune disease progresses even in the presence of this form of TGFβ1. This suggests that IgG-bound TGFβ1 may not be very efficacious in the suppression of systemic autoimmunity. In this regard, T cells have been associated most closely with immunosuppression. Evidence for this comes from studies which show that it is TGFβ secreted by $CD4^+$ and $CD8^+$ T cell clones which is suppressing autoreactivity in adoptive transfer experiments in experimental autoimmune encephalomyelitis (20, 41). In addition, the transfer of hematopoietic and spleen cells from TGFβ1 knockout mice into irradiated nonautoimmune recipients is sufficient to transfer autoantibody production, indicating the importance of cell-delivered TGFβ1 in suppressing autoreactivity (13). It will be important to determine whether IgG-bound TGFβ1 plays a significant role in feedback inhibition of autoreactivity.

Elucidation of the mechanism by which IgG-bound TGFβ1 inhibits phagocyte function will be essential to develop therapeutic interventions which could enhance host defense against infection. Secretion of IgG and active TGFβ1 as a complex could allow IgG to serve as a carrier for active TGFβ1 in vivo and thus prolong its half-life in the circulation. This may account in part for the increased potency of the IgG-bound TGFβ1 complex in vivo. Moreover, the effect of IgG-bound TGFβ1 on phagocyte function could be very different than the effect of the mature form of TGFβ1. IgG could act to deliver TGFβ1 more efficiently to TGFβ receptors on PMN or could even change the surface receptors engaged by TGFβ. This possibility could help to explain some of the discrepancies between known effects of TGFβ1 on phagocytes and what we have observed in MRL/lpr mice. For example, TGFβ1 is known to suppress activation of the respiratory burst and nitric oxide production by activated macrophages and suppression of reactive oxygen and reactive nitrogen metabolites could explain a failure in bactericidal activity. However, phagocytes obtained from peritoneal lavages from MRL/lpr mice after i.p. SA1 challenge are equally able to generate superoxide anion and nitric oxide when stimulated as compared with phagocytes from MRL/n mice (Caver, T.E., and H.D. Gresham, unpublished observation). These data are consistent with other observations indicating increased production of nitric oxide in MRL/lpr mice (42) and suggest that the IgG-bound TGFβ1 present in these mice is not able to suppress formation of these bactericidal metabolites. In addition, TGFβ1 is a known chemoattractant for PMN (43, 44) and it is difficult to reconcile how a cytokine which promotes PMN movement into inflammatory sites could suppress host defense against bacterial infection. One possible explanation for this discrepancy could come from how PMN respond to IgG-bound TGFβ1 versus soluble active TGFβ1.

We believe that the observed defects in PMN phagocytic function in MRL/lpr mice could explain the decreased clearance of the pathogen from the site of infection. To explore this, we examined cytospin preparations of cells from peritoneal lavages obtained 24 h after i.p. SA1 challenge in both MRL/n and MRL/lpr mice. Interestingly, even though the lavage from MRL/lpr mice had $> 10^8$ CFU of SA1/ml and increased numbers of neutrophils, minimal bacteria were associated with the PMN. Conversely, examination of the bacterial lavage obtained from MRL/n mice revealed that phagocytes had surface-associated bacteria (Caver, T.E., and H.D. Gresham, unpublished observations). These data suggest that IgG-bound TGFβ1 inhibits adhesion of bacteria to activated PMN and therefore prevents their uptake and destruction. Currently, studies are underway in our laboratories to determine the molecular mechanism by which IgG-bound TGFβ1 inhibits PMN phagocytic function.

Finally, our results confirm those of others (21–23) which indicate that binding of TGFβ1 to IgG dramatically affects its biologic activity. We have extended this observation to show that TGFβ1 is in the active form inside B cells and plasma cells, that it is complexed to IgG in the active form, that the complex of TGFβ1 bound to IgG is many times more potent than recombinant active TGFβ1 for suppression of PMN function in vitro and host defense against *S. aureus* SA1 infection in vivo, and that this complex can be found in the plasma of some patients with SLE.

Acknowledgments

We thank Fortune Campbell and Candy Trout for excellent technical assistance in these studies. We are grateful to Dr. David Rosen at Celtrix Pharmaceuticals and Dr. Bruce Pratt at Genzyme Corp. for donation of mAb 1D11 and mAb KG7.

This work was supported by the Medical Research Service of the Department of Veterans Affairs (H.D. Gresham and F.X. O'Sullivan), by the American Diabetes Association (H.D. Gresham), and by National Institutes of Health grant CA 49507 (L.I. Gold).

References

1. Staples, P.J., D.N. Gerding, J.L. Decker, and R.S. Gordon. 1974. Incidence of infection in systemic lupus erythematosus. *Arthritis Rheum.* 17:1–10.

2. Rosner, S., E.M. Ginzler, H.S. Diamond, M. Weiner, M. Schlesinger, J.F. Fries, C. Wasner, T.A. Medsger, Jr., G. Ziegler, J.H. Klippel, et al. 1982. A multicenter study of outcome in systemic lupus erythematosus. II. Causes of death. *Arthritis Rheum.* 25:612–617.

3. Nived, O., G. Sturfelt, and F. Wollheim. 1985. Systemic lupus erythematosus and infection: a controlled and prospective study including an epidemiologic group. *Q. J. Med.* 55:271–287.

4. Abu-Shakra, M., B. Urowits, D. Gladman, and J. Gough. 1995. Mortality studies in systemic lupus erythematosus. Results from a single center. I. Causes of death. *J. Rheumatol.* 22:1259–1264.

5. Reveille, J., A. Bartolucci, and G.S. Alarcon. 1990. Prognosis and systemic lupus erythematosus. *Arthritis Rheum.* 33:37–48.

6. Pistiner, M., D.J. Wallace, S. Nessin, A.L. Metzger, and J.R. Klineberg. 1991. Lupus erythematosus in the 1980's: a survey of 570 patients. *Semin. Arthritis Rheum.* 21:55–64.

7. Dale, D., and R. Petersdorf. 1973. Corticosteroids and infectious diseases. *Med. Clin. North Am.* 57:1277–1287.

8. Watanabe-Duffy, K.N., D.M. Duffy, and D.D. Gladman. 1991. Infection and disease activity in systemic lupus erythematosus: a review of hospitalized patients. *J. Rheumatol.* 18:1180–1184.

9. Gresham, H.D., C.J. Ray, and F. X. O'Sullivan. 1991. Defective neutrophil function in the autoimmune mouse strain MRL/lpr: potential role of transforming growth factor-β. *J. Immunol.* 146:3911–3921.

10. Lowrance, J., F. O'Sullivan, T. Caver, W. Waegell, and H.D. Gresham. 1994. Spontaneous elaboration of transforming growth factor-β suppresses host defense against bacterial infection in autoimmune MRL/lpr mice. *J. Exp. Med.* 180:1693–1703.

11. Wahl, S. 1994. Transforming growth factor β. The good, the bad, the

ugly. *J. Exp. Med.* 180:1587–1590.

12. Dang, H., A.G. Geiser, J.J. Letterio, T. Nakabayashi, L. Kong, G. Fernandes, and N. Talal. 1995. SLE-like autoantibodies and Sjogren's syndrome-like lymphoproliferation in TGFβ knockout mice. *J. Immunol.* 155: 3205–3212.

13. Yaswen, L., A.B. Kulkarni, T. Fredrickson, B. Mittleman, R. Schiffmann, S. Payne, G. Longenecker, E. Mozes, and S. Karlsson. 1996. Autoimmune manifestations in the transforming growth factor-β1 knockout mouse. *Blood.* 87:1439–1445.

14. Sanderson, N., V. Factor, P. Nagy, J. Kopp, P. Kondaiah, L. Wakefield, A. Roberts, M. Sporn, and S. Thorgeirsson. 1995. Hepatic expression of mature transforming growth factor β1 in transgenic mice results in multiple tissue lesions. *Proc. Natl. Acad. Sci. USA.* 92:2572–2576.

15. Raz, E., M. Lotz, S. Baird, R. Eisenberg, I. Wicks, and D. Carson. 1993. Modulation of murine systemic lupus erythematosus by cytokine gene delivery. *Arthritis Rheum.* 36:S91*a*. (Abstr.)

16. Kuruvilla, A.P., R. Shah, G.M. Hochwald, H.D. Liggitt, M. Palladino, and G.J. Thorbecke. 1991. Protective effect of transforming growth factor β1 on experimental autoimmune diseases in mice. *Proc. Natl. Acad. Sci. USA.* 88: 2918–2921.

17. Border, W.A., S. Okuda, L. Languino, M.B. Sporn, and E. Ruoslahti. 1990. Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1. *Nature (Lond.).* 346:371–374.

18. Brandes, M.E., J.B. Allen, Y. Ogawa, and S.M. Wahl. 1991. Transforming growth factor β1 suppresses acute and chronic arthritis in experimental animals. *J. Clin. Invest.* 87:1108–1113.

19. Allen, J.B., C.L. Manthey, A.R. Hand, K. Ohura, L. Ellingsworth, and S.M. Wahl. 1990. Rapid onset synovial inflammation and hyperplasia induced by transforming growth factor β. *J. Exp. Med.* 171:231–247.

20. Chen, Y., V.K. Kuchroo, J.-I. Inobe, D.A. Hafler, and H.L. Weiner. 1994. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science (Wash. DC).* 265:1237–1240.

21. Stach, R.M., and D.A. Rowley. 1993. A first or dominant immunization. II. Induced immunoglobulin carries transforming growth factor β and suppresses cytolytic T cell responses to unrelated alloantigens. *J. Exp. Med.* 178: 841–852.

22. Bouchard, C., A. Galinha, E. Tartour, W.H. Fridman, and C. Sautes. 1995. A transforming growth factor β-like immunosuppressive factor in immunoglobulin G-binding factor. *J. Exp. Med.* 182:1717–1726.

23. Rowley, D.A., E.T. Becken, and R.M. Stach. 1995. Autoantibodies produced spontaneously by young lpr mice carry transforming growth factor β and suppress cytotoxic T lymphocyte responses. *J. Exp. Med.* 181:1875–1880.

24. Satoh, M., A. Kumar, Y.S. Kanwar, and W.H. Reeves. 1995. Anti-nuclear antibody production and immune-complex glomerulonephritis in BALB/c mice treated with pristane. *Proc. Natl. Acad. Sci. USA.* 92:10934–10939.

25. Dasch, J.R., D.R. Pace, W. Waegell, D. Inenaga, and L. Ellingsworth. 1989. Monoclonal antibodies recognizing transforming growth factor-β. Bioactivity neutralization and transforming growth factor β2 affinity purification. *J. Immunol.* 142:1536–1541.

26. Danielpour, D. 1993. Improved sandwich enzyme-linked immunoabsorbent assays for transforming growth factor β1. *J. Immunol. Methods.* 158:17–25.

27. Schultz-Cherry, S., S. Ribeiro, L. Gentry, and J.E. Murphy-Ullrich. 1994. Thrombospondin binds and activates the small and large forms of latent transforming growth factor β in a chemically defined system. *J. Biol. Chem.* 269: 26775–26781.

28. Pelton, R.W., B. Saxena, M. Jones, H.L. Moses, and L.I. Gold. 1991. Immunohistochemical localization of TGFβ1, TGFβ2, and TGFβ3 in the mouse embryo. Expression patterns suggest multiple roles during embryonic development. *J. Cell Biol.* 115:1091–1105.

29. Nunes, I., R.L. Shapiro, and D.B. Rifkin. 1995. Characterization of latent TGF-β activation by murine peritoneal macrophages. *J. Immunol.* 155: 1450–1459.

30. Goddard, D.H., A.P. Kirk, J.R. Kirwan, G.D. Johnson, and E.J. Holborow. 1984. Impaired polymorphonuclear leukocyte chemotaxis in rheumatoid arthritis. *Ann. Rheum. Dis.* 43:151–156.

31. Wandall, J.H. 1985. Leukocyte function in patients with rheumatoid arthritis: quantitative in vivo leukocyte mobilization and in vitro functions of blood and exudate leukocytes. *Ann. Rheum. Dis.* 44:694–700.

32. Wheat, L.J. 1980. Infection and diabetes mellitus. *Diabetes Care.* 3:187–197.

33. Wilson, R.M., and W.G. Reeves. 1986. Neutrophil phagocytosis and killing in insulin-dependent diabetes. *Clin. Exp. Immunol.* 63:478–484.

34. Witt, D.J., D.E. Craven, and W.R. McCabe. 1987. Bacterial infections in adult patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Am. J. Med.* 82:900–906.

35. Ellis, M., S. Gupta, and S. Galant. 1988. Impaired neutrophil function in patients with AIDS or AIDS-related complex: a comprehensive evaluation. *J. Infect. Dis.* 158:1268–1276.

36. Lim, S.H., F.J. Giles, M.P. Smith, and A.H. Goldstone. 1991. Bacterial infections in lymphoma patients treated with recombinant interleukin-2. *Acta Haematol. (Basel).* 85:135–138.

37. Snydman, D.R., B. Sullivan, M. Gill, J.A. Gould, D.R. Parkinson, and M.B. Atkins. 1990. Nosocomial sepsis associated with interleukin-2. *Ann. Intern. Med.* 112:102–107.

38. Puolakkainen, P., D. Twardzik, J. Ranchalis, M. Moroni, J. Mandeli, and P.A. Paciucci. 1995. Increase of plasma transforming growth factor beta (TGFβ) during immunotherapy with IL-2. *Cancer Invest.* 13:583–589.

39. Allen, J.B., H.L. Wong, P.M. Guyre, G.L. Simon, and S.M. Wahl. 1991. Association of circulating FcγRIII-positive monocytes in AIDS patients with elevated levels of transforming growth factor-β. *J. Clin. Invest.* 87:1773–1779.

40. Lotz, M., and P. Seth. 1993. TGFβ and HIV infection. *Ann. NY Acad. Sci.* 685:501–511.

41. Weiner, H.L., J. Inobe, V. Kuchroo, and Y. Chen. 1996. Induction and characterization of TGF-beta secreting Th3 cells. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 10:2558*a*. (Abstr.)

42. Huang, F.P., G.J. Feng, G. Lindop, D.I. Scott, and F.Y. Liew. 1996. The role of interleukin 12 and nitric oxide in the development of spontaneous autoimmune disease in MRL/MP-lpr/lpr mice. *J. Exp. Med.* 183:1447–1459.

43. Brandes, M.E., U.E. Mai, K. Ohura, and S.M. Wahl. 1991. Human neutrophils express type I TGFβ receptors and chemotax to TGFβ. *J. Immunol.* 147:1600–1606.

44. Parekh, T., B. Saxena, J. Reibman, B.N. Cronstein, and L. Gold. 1994. Neutrophil chemotaxis in response to TGFβ isoforms (TGFβ1, TGFβ2, TGFβ) is mediated by fibronectin. *J. Immunol.* 152:2456–2466.