Spontaneous Elaboration of Transforming Growth Factor β Suppresses Host Defense against Bacterial Infection in Autoimmune MRL/lpr Mice

By Jon H. Lowrance,*‡∥ Frank X. O'Sullivan,*§ Tony E. Caver,*∥ Wendy Waegell,¶ and Hattie D. Gresham*‡∥

From the *Research Service, Harry S. Truman Veterans Affairs Medical Center, Columbia, Missouri 65201; Departments of ‡Pharmacology, §Medicine, and ∥Molecular Microbiology and Immunology, University of Missouri-Columbia, Columbia, Missouri 65212; and ¶Celtrix Pharmaceuticals, Santa Clara, California 95054

Summary

Infection with gram-negative and gram-positive bacteria remains a leading cause of death in patients with systemic lupus erythematosis (SLE), even in the absence of immunosuppresive therapy. To elucidate the mechanisms that underly the increased risk of infection observed in patients with systemic autoimmunity, we have investigated host defense against bacterial infection in a murine model of autoimmunity, the MRL/Mp-lpr/lpr (MRL/lpr) mouse. Our previous study implicated transforming growth factor β (TGF-β) in a novel acquired defect in neutrophil function in MRL/lpr but not congenic MRL/Mp-+/+ (MRL/n) mice (Gresham, H.D., C.J. Ray, and F.K. O'Sullivan. 1991. J. Immunol. 146:3911.) We hypothesized from these observations that MRL/lpr mice would have defects in host defense against bacterial infection and that they would have constitutively higher local and systemic levels of active TGF-β which would be responsible, at least in part, for the defect in host defense. We show in this paper that spontaneous elaboration of active TGF-β adversely affects host defense against both gram-negative and gram-positive bacterial infection in MRL/lpr mice. Our data indicate that MRL/lpr mice, as compared with congenic MRL/n mice, exhibit decreased survival in response to bacterial infection, that polymorphonuclear leukocytes (PMN) from MRl/lpr mice fail to migrate to the site of infection during the initial stages of infection, that MRL/lpr mice have a significantly increased bacterial burden at the site of infection and at other tissue sites, and that this increased bacterial growth occurs at a time (>20 h after infection) when PMN influx is greatly enhanced in MRL/lpr mice. Most intriguingly, the alteration in PMN extravasation during the initial stages of infection and failure to restrict bacterial growth in vivo could be duplicated in MRL/n mice with a parenteral injection of active TGF-β1 at the time of bacterial challenge. Moreover, these alterations in host defense, including survival in response to lethal infection, could be ameliorated in MRL/lpr mice by the parenteral administration of a monoclonal antibody that neutralizes the activity of TGF-β. These data indicate that elaboration of TGF-β as a result of autoimmune phenomenon suppresses host defense against bacterial infection and that such a mechanism could be responsible for the increased risk of bacterial infection observed in patients with autoimmune diseases.

Infection with gram-negative and gram-positive bacteria remains a leading cause of death in patients with systemic lupus erythematosus (SLE)[1] (1–4). However, the factors underlying the morbidity and mortality caused by local and sys-

temic infection observed in human autoimmune diseases are not well defined. Although this increased risk of infection has been attributed primarily to therapeutic immunosuppression, lethal bacterial infection does occur in the absence of immunosuppressive therapy (1–3). In this regard, defects in neutrophil (PMN) function have been investigated as possible contributors to infection in patients with SLE (5–7). To elucidate the relationship of putative defects in PMN function to the risk of infection in patients with autoimmune

---

[1] Abbreviations used in this paper: EAE, experimental autoimmune encephalitis; K1+, E. coli K1+; MRL/lpr, MRL/Mp-lpr/lpr; MRL/n, MRL/Mp-+/+; RA, rheumatoid arthritis; SA1, S. aureus SA1; SLE, systemic lupus erythematosis.

diseases, we investigated PMN function in a murine model of systemic autoimmunity, the MRL/Mp-lpr/lpr (MRL/lpr) mouse (8–10). Our previous work indicated that PMN from MRL/lpr mice, but not congenic MRL/Mp-+/+ (MRL/n) mice, exhibited defects in extravasation to a site of inflammation and in amplification of phagocytic function (11). Our data implicated TGF-β in the acquisition of these defects. From these observations, we hypothesized that MRL/lpr mice would have defects in host defense against bacterial infection and that they would have constitutively higher local or systemic levels of active TGF-β which would be responsible, at least in part, for the defect in host defense.

TGF-β is a multifunctional cytokine with a regulatory role in a broad spectrum of biological processes (reviewed in 12). It is a vital bifunctional immune modulator, exhibiting both proinflammatory (12–14) and immunosuppressive properties (13). The immunosuppressive activities of TGF-β have provided the therapeutic basis for its use in the treatment of several nonspontaneous animal models of autoimmune disease, including experimental autoimmune encephalitis (EAE) (15–17), streptococcal cell wall–induced arthritis (18), and collagen-induced arthritis (15). In fact, in vivo production of TGF-β may be responsible for the spontaneous resolution of EAE observed in some animals (19). In contrast to its therapeutic activity in experimental autoimmune disease, mounting evidence in murine models of leishmaniasis and trypanosomiasis indicates that enhanced production of active TGF-β correlates strongly with susceptibility to infection (20, 21). In addition, HIV infection induces elevated levels of TGF-β which have been implicated in the immunosuppression observed in patients with AIDS (22, 23). To date, no studies exist that have examined the effect of spontaneous production of active TGF-β, which may occur in human autoimmune diseases (24), on the susceptibility to either gram-positive or gram-negative bacterial infection.

Therefore, the purpose of the present work is to test our hypothesis that spontaneous elaboration of active TGF-β in autoimmune MRL/lpr mice is causally related to an increased susceptibility to bacterial infection. Here, we show that MRL/lpr mice have a significantly increased risk of lethal infection upon challenge with bacterial strains Escherichia coli K1+ (K1+) and Staphylococcus aureus SA1 (SA1) and that treatment of MRL/lpr mice with anti-TGF-β significantly ameliorates their host defense defect. These data suggest that use of TGF-β antagonists in patients with autoimmune diseases may represent an important therapeutic modality for augmentation of host defense against infection with common bacterial pathogens.

Materials and Methods

Mice. MRL/lpr and MRL/n mice were obtained originally from The Jackson Laboratory (Bar Harbor, ME) and were bred and maintained in microisolator housing units (Lab Products, Inc., Maywood, NJ) under specific pathogen-free conditions. The pathogen-free status of the colony was routinely monitored by complete health surveillance including necrospy examinations, appropriate cultures, and serologic studies by the Research Animal Diagnostic and In-

vestigation Laboratory of the University of Missouri College of Veterinary Medicine-Columbia (Columbia, MO). The surveillance showed no evidence of infection with naturally occurring murine pathogens, including Sendai virus and mouse hepatitis virus.

Autoimmune and Host Defense Parameters. Age-matched male MRL/n and MRL/lpr mice were used in this study to avoid any influence of gender on the host response to infection. The autoimmune status and host defense status of 10- and 16-wk-old male MRL/n and MRL/lpr mice were assessed as follows: blood urea nitrogen (BUN), IgG antibodies to double-stranded (ds)DNA by ELISA, and spleen weights as described (10); serum IgG levels by RID (The Binding Site, San Diego, CA), and serum C3 levels by RID (The Binding site). Sera to be tested were collected from five mice in each group by cardiac puncture while under the anesthetic effects of methoxyflurane (Pitman-Moore, Mundelein, IL). As has been observed in female MRL/lpr mice (10), serum IgG levels, spleen weights, and IgG antibodies to dsDNA were all significantly higher in 10- and 16-wk-old male MRL/lpr mice than in age- and sex-matched MRL/n mice ($p < 0.01$, Mann-Whitney test for nonparametrics). In contrast, the serum BUN and C3 levels were not significantly different in 10- and 16-wk-old male MRL/lpr mice from those of age- and sex-matched MRL/n mice and are generally not observed to be different until 20–24 wk of age (8, 10) (data not shown). The percentage of granulocytes in the bone marrow of 12-wk-old male MRL/lpr and MRL/n mice was assessed by fluorescence flow cytometry. Femurs were removed aseptically and flushed twice with HBSS (GIBCO BRL, Gaithersburg, MD) to obtain bone marrow leukocytes (BML). BML were stained by indirect immunofluorescence as described (11) with rat IgG2b anti–mouse granulocyte Gr-1 (clone RB6-8C5; PharMingen, San Diego, CA) or rat IgG2b as a control. Both MRL/n and MRL/lpr mice had equivalent numbers of bone marrow granulocytes (77.2 ± 2.0 and 82.8 ± 1.6%, respectively). The majority of experiments were performed with 10–12-wk-old male mice.

Microorganisms. E. coli O18:K1:H7, strain Bort and E. coli xyl were obtained from Dr. A. S. Cross (Walter Reed Army Institute of Research, Washington, DC). S. aureus SA1 was obtained from Dr. J. Lee (Harvard University, Cambridge, MA) and S. aureus ST5, a microencapsulated strain was obtained from D. L. Baddour (University of Tennessee, Knoxville, TN). Strains K1+ and SA1 are pathogenic and both strains are encapsulated (25–27). K1+ was grown in trypticase soy broth (Difco Laboratories, Detroit, MI) overnight at 37°C and SA1 was grown on blood agar (Remel, Lenexa, KS) overnight at 37°C. The microorganisms were suspended in pyrogen-free, sterile normal saline (Kendall McGaw, Irvine, CA) and adjusted to the desired inoculum spectrophotometrically at 530 nm before injection. To maintain virulence, bacteria were passaged in an MRL/lpr mouse within 48 h before injection into the experimental animals.

Bacterial Challenge. Mice were injected either intraperitoneally or subcutaneously with bacteria that had been prepared as indicated. The inoculum used for bacterial challenge was confirmed by serial dilutions in sterile normal saline and each dilution was counted by a spread-plate method onto the appropriate agar media for the microorganism used in the experiment. Once injected, mice were observed over a 14-d period and the results expressed as the percent of mice surviving the bacterial challenge. Statistical significance was evaluated using the Fisher's exact test.

In addition to lethality, host susceptibility to bacterial infection was measured by examining viable bacterial counts in a lavage of the peritoneum and in liver and spleen homogenates obtained at various time points after intraperitoneal bacterial challenge. Mice were killed by cervical dislocation while under the anesthetic effects

of methoxyflurane, after which the peritoneum was lavaged with 5 ml of sterile HBSS. Recovered lavage fluid from each mouse was serially diluted and aliquots of each dilution were spread-plated onto appropriate agar medium to obtain bacterial counts. Percentage and numbers of PMN/ml of peritoneal lavage were determined as described previously (11). After peritoneal lavage, the livers and spleens were resected and homogenized in sterile glass tissue grinders with 1 ml of HBSS. An aliquot of each homogenate was serially diluted and dilutions were spread-plated onto appropriate media to obtain approximate bacterial counts. Statistical differences in bacterial CFU were determined by the Mann-Whitney U test for nonparametrics.

TGF-β Assay. Splenic culture supernatants for assay of TGF-β were generated as follows: spleens from 10- and 16-wk-old male MRL/n and MRL/lpr mice were removed aseptically, dispersed into media, and washed with 10 mM EDTA to deplete platelets (28). The cells were suspended in RPMI supplemented with Nutridoma-SP (Boehringer Mannheim Corp., Indianapolis, IN) at 10^7/ml and incubated in 24-well plates at 37°C in 5% CO_2. After 24 h, the cells were removed by centrifugation and the supernatants assayed directly for measurement of active TGF-β or acidified to measure total TGF-β. Levels of active TGF-β were measured in the peritoneal lavage samples generated after intraperitoneal challenge with SA1. Lavage samples were centrifuged at 12,500 g for 10 min and sterile-filtered before assessment of TGF-β. TGF-β was measured with a bioassay using CCL-64 mink lung cells (American Type Culture Collection, Rockville, MD) (29). Briefly, subconfluent CC1-64 cells were trypsinized and plated onto 96-well tissue culture plates at 5 × 10^4 cells/well in 100 μl of DMEM medium supplemented with 10% FCS 10 mM Hepes, nonessential amino acids, glutamine, and gentamycin. Cells were incubated at 37°C in 10% CO_2 for 1 h to allow sufficient time for cells to adhere. Experimental samples or TGF-β standards diluted in complete DMEM without FCS were added in 100 μl aliquots to a final volume of 200 μl/well. The mixtures were incubated at 37°C in 10% CO_2 for an additional 21 h after which the cells were pulsed with 0.5 μCi/well [^{3}H]thymidine for 2 h, washed, and harvested onto filter disks for counting in a liquid scintillation counter (model LS6000IC, Beckman Instruments, Palo Alto, CA). Growth inhibition was calculated as follows: percent growth inhibition = 100 × [1 − (cpm sample/cpm control)], where cpm control represents baseline thymidine incorporation in the absence of added TGF-β. Concentrations were obtained by comparison with a standard curve derived from purified human platelet TGF-β1 (R & D Systems, Inc., Minneapolis, MN). The data were analyzed by the Mann-Whitney test for nonparametrics. This assay predominately measures TGF-β because treatment of splenic supernatants with either a polyclonal rabbit IgG anti-TGF-β (R & D Systems, Inc.) or with a monoclonal murine IgG1 anti-TGF-β (Clone 1D11.16) neutralized 75–80% of the growth inhibiting activity.

Treatment with TGF-β and Anti-TGF-β. Mice were under the anesthetic effects of methoxyflurane during the tail vein injections. Purified human platelet TGF-β1 (R & D Systems) was activated by reconstituting in sterile 4 mM HCl containing 0.1% human serum albumin overnight at 4°C. The activated TGF-β1 was neutralized and adjusted with sterile normal saline to 2 μg/ml. The vehicle control was normal saline which was treated in the same manner as the TGF-β1. MRL/n mice were injected with 300 ng of active TGF-β1 or vehicle control immediately before intraperitoneal challenge with SA1. MRL/lpr mice were injected via the tail vein with 166 μg of murine IgG1 monoclonal anti-TGF-β, clone 1D11.16, prepared as described (30), or murine IgG1 (The Binding Site) as a control. mAb 1D11.16 was able to neutralize murine TGF-β assayed by the mink lung cell assay described above. Before injection,

both antibodies were subjected to buffer exchange with pyrogen-free, sterile normal saline by dilution and concentration 3× with Centricon-30 microconcentrators (Amicon Corp., Beverly, MA). The antibody concentration was adjusted to 1 mg/ml and assayed for the presence of endotoxin using an E-toxate Kit (Sigma Chemical Co., St. Louis, MO). The level of endotoxin was below 0.025 IU/ml of injected material. Immediately after the antibody injections, the mice were challenged intraperitoneally with SA1.

Statistical Analysis. Assistance with statistical analyses was provided by Dr. John Hewett (University of Missouri-Columbia, Columbia, MO).

Results

MRL/lpr Mice Are More Susceptible to the Lethal Effects of K1+ and SA1 Than Are MRL/n Mice. Our previous data indicated a novel acquired defect in PMN function in MRL/lpr, but not congenic MRL/n mice (11). We hypothesized that such a PMN defect would make these mice more susceptible to infection. To investigate this possibility, male MRL/lpr and MRL/n mice were injected with either SA1 or K1+. These bacterial strains are pathogenic for mice and rats (25–27) and infections with these organisms are representative of those observed in humans with defects in PMN function (31) and site SLE (1–3). Bacterial challenge was performed in male MRL/n and MRL/lpr mice at two different sites of injection (subcutaneous and intraperitoneal) and with different inocula (CFU) in 10–20-wk-old animals. After observation for 14 d, the percentage of surviving animals was assessed.

As shown in Fig. 1 *A*, the survival of 10-wk-old male MRL/n mice (100%) was significantly greater than age- and sex-matched MRL/lpr mice (25%) after an intraperitoneal injection with 3.2 × 10^4 CFU of SA1 (p <0.005, Fisher's exact test). Similar results were obtained when 20-wk-old male mice were challenged intraperitoneally with 3.7 × 10^3 CFU of SA1 (Fig. 1 *B*). MRL/n mice had a survival rate of 84% whereas MRL/lpr mice had a survival rate of 17% (Fig. 1 *B*; p <0.05). These data indicate that MRL/lpr mice have a significant risk of lethal infection as compared with
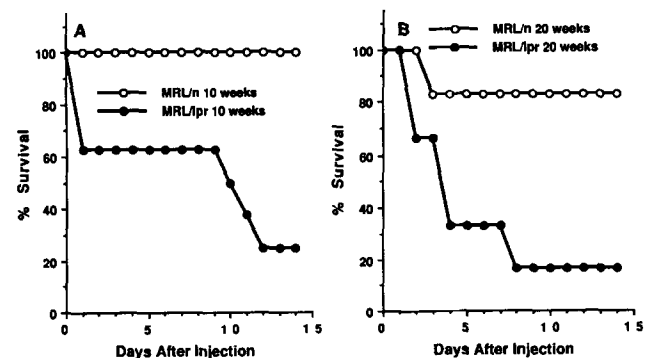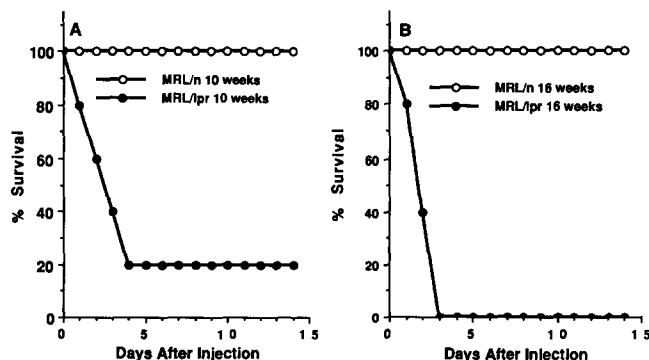

Figure 1. Effect of *S. aureus* SA1 injected i.p. on the survival of (*A*) 10-wk-old male MRL/n (*n* = 8) and MRL/lpr mice (*n* = 8) and (*B*) 20-wk-old male MRL/n (*n* = 6) and MRL/lpr mice (*n* = 6). Animals were injected with 3.2 × 10^4 CFU of SA1 (*A*) or 3.7 × 10^3 CFU (*B*). Survival is significantly decreased for male MRL/lpr mice at both 10 wk (p <0.005, Fisher's exact test) and 20 wk of age (p <0.05, Fisher's exact test).

MRL/n mice and that this risk is present at an age (10–12 wk) when their renal function, complement component C3 levels, and numbers of granulocytes are not significantly different from age- and sex-matched MRL/n mice (see Materials and Methods for discussion of these parameters).

To demonstrate that an increased susceptibility of bacterial infection in MRL/lpr mice was not limited to gram-positive bacteria, we next investigated the effect of a gram negative bacteria, K1+, on the survival of these murine strains. As shown in Fig. 2 A, the survival of 10-wk-old MRL/n mice (100%) was significantly greater than age- and sex-matched MRL/lpr mice (20%) after intraperitoneal injection of 2 × 10^3 CFU of K1+ (p <0.05). Identical results were obtained when 1.2 × 10^4 CFU of K1+ was injected subcutaneously into 16-wk-old male mice (100% survival for MRL/n vs. 0% survival for MRL/lpr, p <0.001, Fig. 2 B). These data indicate that MRL/lpr mice, irrespective of age, route of administration, or type of bacteria used, were significantly more susceptible to lethal bacterial infection when compared with MRL/n mice.

One possible explanation for these data could be that endotoxins or other bacterial products were primarily responsible for decreased survival of MRL/lpr mice rather than infection and colonization by the microorganism. To address this possibility, two other strains were tested in MRL/lpr and MRL/n mice. A nonencapsulated but LPS+ strain, E. coli xyl, was injected subcutaneously (6 × 10^4 CFU) into six MRL/n and six MRL/lpr mice. After 14 d, survival was 100% in both strains of mice (data not shown). In addition, S. aureus ST5, a microencapsulated strain, was injected intraperitoneally (10^7 CFU) into five mice from each strain. After 14 d, survival was 100% in both strains of mice (data not shown). These data suggest that the decreased survival observed in MRL/lpr mice in Figs. 1 and 2 was due to the overall virulence of the bacteria and the host susceptibility to bacterial infection.

Although the data in Figs. 1 and 2 reflect an increased risk of lethal infection in MRL/lpr mice as compared with MRL/n
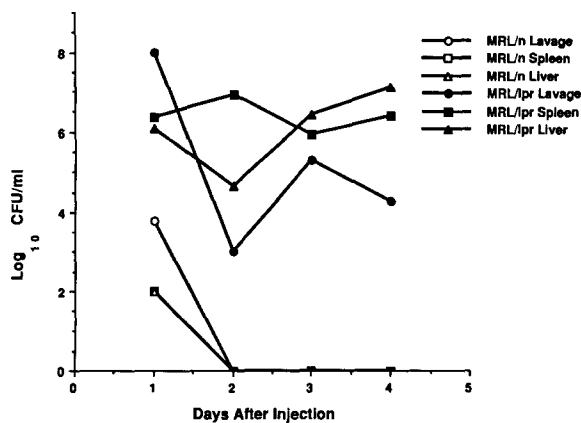
mice, differences in survival between the two strains were not observed at every concentration of CFU examined. For example, neither MRL/lpr nor MRL/n mice survived injection of 10^6 or greater CFU of either K1+ or SA1 (data not shown). In addition, both MRL/n and MRL/lpr mice survived injection with 10^2 CFU or less of either microorganism. Thus, the increased risk of lethal infection observed in MRL/lpr mice is dependent on the concentration of microorganism used to challenge the animals.

Comparison of MRL/n and MRL/lpr Mice for Bacterial Burden and PMN Extravasation after Intraperitoneal SA1 Challenge. Mortality after serious infection is likely the result of complex events involving both the host response to the pathogen and the ability of the microorganism to evade host defenses, proliferate, and colonize beyond the initial site of infection. The decreased survival of the MRL/lpr mice in response to bacterial challenge could result from altered host responses (i.e., overproduction of IL-1, etc.) instead of the ability of the pathogen to evade host defenses and proliferate. Therefore, to more precisely compare the risk of bacterial infection in MRL/lpr and MRL/n mice, we evaluated over time the recovery of viable bacteria from the peritoneum as well as other tissue sites (liver and spleen) following intraperitoneal bacterial challenge. For these and subsequent experiments, 10–12-wk-old male MRL/n and MRL/lpr mice were used. In addition, all subsequent bacterial challenge experiments were performed with SA1 because it retained its pathogenic phenotype in propagative culture more reliably than the K1+ strain.

When bacterial burden was assessed over 4 d after intraperitoneal injection of SA1, MRL/n mice cleared the microorganisms within 48 h from the peritoneum, liver, and spleen. However, the MRL/lpr mice continued to have a large burden of bacteria in the peritoneum, liver, and spleen (range: 10^4 to 10^7 CFU) during this time period (Fig. 3). These data

Figure 2. Effect of *E. coli* K1+ injected intraperitoneally (*A*) and subcutaneously (*B*) on the survival of (*A*) 10-wk-old male MRL/n (*n* = 6) and MRL/lpr mice (*n* = 5) and (*B*) 16-wk-old male MRL/n (*n* = 6) and MRL/lpr mice (*n* = 5). Animals were injected with 2 × 10^3 CFU of K1+ (*A*) or 1.2 × 10^4 CFU (*B*). Survival is significantly decreased for male MRL/lpr mice at both 10 wk (p <0.05, Fisher's exact test) and 16 wk of age (p <0.001, Fisher's exact test).

Figure 3. Comparison of MRL/n (*open symbols*) and MRL/lpr mice (*solid symbols*) for the recovery for viable bacteria 1–4 d after intraperitoneal challenge with 3.2 × 10^4 CFU of SA1. The data are depicted as the $\log_{10}$ CFU/ml of a 5-ml lavage of the peritoneal cavity (*circles*) or per ml of a 1 ml homogenate of either the liver (*triangles*) or spleen (*squares*). MRL/n mice cleared the bacterial challenge from the lavage, liver, and spleen within 2 d whereas the MRL/lpr mice were unable to restrict the growth of SA1.

indicate that this microorganism was able to overcome host defenses in MRL/lpr mice, increase in number over the original inoculum injected, and colonize not only the peritoneum, but other organs as well. We conclude from these observations that MRL/lpr mice were unable to adequately clear an infection or contain the infection to a local site.

Moreover, the results of these experiments indicated that the host's response within the first 24 h after bacterial challenge was critical in the ability of MRL/n and MRL/lpr mice to survive. Therefore, we examined the bacterial burden in the peritoneal lavage 4 and 24 h after intraperitoneal challenge with SA1. Because we observed previously that MRL/lpr PMN failed to extravasate into thioglycollate stimulated peritoneal exudates (11), we also assessed the percentage and number of PMN in the peritoneal exudate. As shown in Fig. 4 *A*, MRL/lpr PMN failed to extravasate into the peritoneum, as compared with MRL/n PMN, during the first 4 h after intraperitoneal bacterial challenge. The differences between the two strains in percentage of PMN were reflected also in the numbers of PMN/ml of peritoneal lavage (Fig. 4 *B*). Both the percentage of PMN and the number of PMN/ml of lavage were significantly lower in MRL/lpr mice ($p < 0.03$ and $p < 0.03$, respectively) (Fig. 4, *A* and *B*). In addition, the total cell counts in the lavage were significantly lower in the MRL/lpr mice (data not shown). MRL/n mice had greater than three times the number of PMN in the peritoneal lavage as did MRL/lpr mice. No significant differences in the recovery of viable bacteria from the peritoneal lavage were observed at this early time point. (Fig. 4 *C*).

It is important to note that 24 h after bacterial challenge, both the percentage and the number of PMN present in the peritoneal lavage of MRL/lpr mice had increased and was significantly greater than the percentage and number of PMN present in MRL/n mice ($p < 0.002$ and $p < 0.001$, respectively) (Fig. 4, *A* and *B*). In addition, the total cell counts in the lavage were significantly higher in the MRL/lpr mice (data not shown). Therefore, by 24 h, the percentage of PMN present in the peritoneum of MRL/lpr mice was continuing to increase at a time when the percentage of PMN present in the peritoneum of MRL/n mice was abating, consistent

with a pattern of resolving inflammation (Fig. 4 *A*). In fact, the number of PMN/ml increased 35-fold in the MRL/lpr mice whereas it increased only 2.5-fold in MRL/n mice (Fig. 4 *B*). Moreover, the recovery of viable bacteria from the peritoneal lavage 24 h after intraperitoneal challenge was significantly different between the two strains ($p < 0.001$) (Fig. 4 *C*). The MRL/lpr mice had 44-fold more bacteria in the lavage than did the MRL/n mice. In addition, MRL/lpr mice had significantly greater numbers of CFU in the liver ($p < 0.002$) and the spleen ($p < 0.04$) in comparison with MRL/n mice (data not shown). Thus, 24 h after the bacterial challenge, the microorganisms were proliferating in the peritoneum of the MRL/lpr mice even though the percentage and number of PMN had increased. Comparison of bacterial burden 4 and 24 h after intraperitoneal challenge shows that MRL/lpr mice are unable to restrict the growth of the microorganism over this 20-h period as compared with MRL/n mice (Fig. 4 *C*). These data demonstrate that MRL/lpr PMN are impaired in their ability to extravasate to a site of infection during the initial stages of infection and that they are not able to adequately contain an infection once present at the site.

MRL/lpr Mice Exhibit Significantly Elevated Levels of Active and Total TGF-β Compared with MRL/n Mice. Our previous data implicated TGF-β in the acquisition of the defective PMN function observed in MRL/lpr mice (11). We investigated the spleen as a source of potentially activated cells with known capability for producing TGF-β (13) by assessing the levels of both active and total TGF-β in supernatants of platelet-depleted, resting spleen cell cultures from 10- and 16-wk-old male MRL/n and MRL/lpr mice. As shown in Fig. 5, MRL/lpr mice released significantly higher levels of both active and total TGF-β in 24-h supernatants of resting spleen cell cultures as compared with MRL/n mice at both 10 wk ($p < 0.05$ and $p < 0.05$, respectively) and 16 wk of age ($p < 0.05$ and $p < 0.05$, respectively, Student's t test). Similar results were obtained after 72 h of culture. These data demonstrate that unstimulated MRL/lpr spleen cells produce elevated levels of active TGF-β and that this phenomenon increases in magnitude with age.
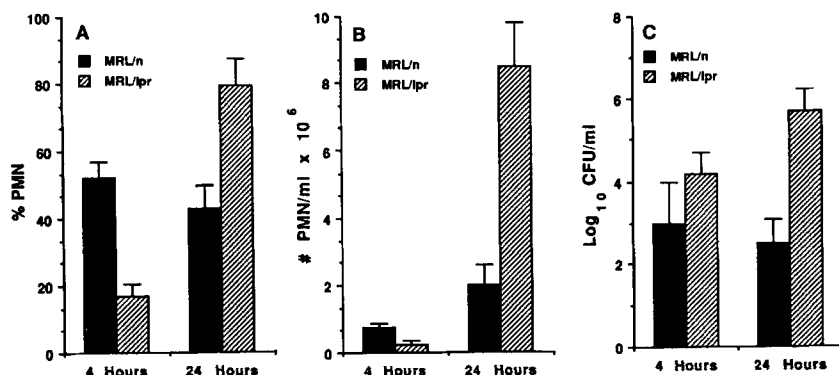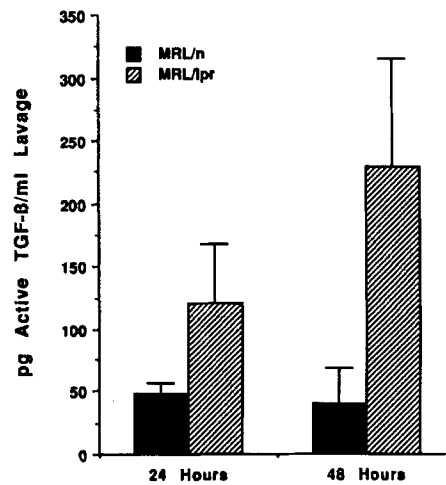

Figure 4. Comparison of MRL/n and MRL/lpr mice for (*A*) percent PMN, (*B*) PMN number, and (*C*) $\log_{10}$ CFU/ml of a lavage of the peritoneal cavity and 4 and 24 h after intraperitoneal challenge with 4.8×10^4 CFU of SA1. At 4 h, the percent PMN and PMN number/ml, but not the CFU/ml, were significantly different between MRl/n ($n = 4$) and MRL/lpr mice ($n = 4$) ($p < 0.03$ and $p < 0.03$, respectively, Mann-Whitney U test). At 24 h, the percent PMN, PMN number/ml, and the CFU/ml were significantly different between MRL/n ($n = 13$) and MRL/lpr mice ($n = 14$) ($p < 0.002$, $p < 0.001$, and $p < 0.001$, respectively, Mann-Whitney U test). MRL/lpr PMN are impaired in their ability to extravasate to a site of infection during the initial stages of infection (4 h) and they are not able to adequately restrict the growth of the microorganism once present at the site, resulting in significantly increased bacterial burden.
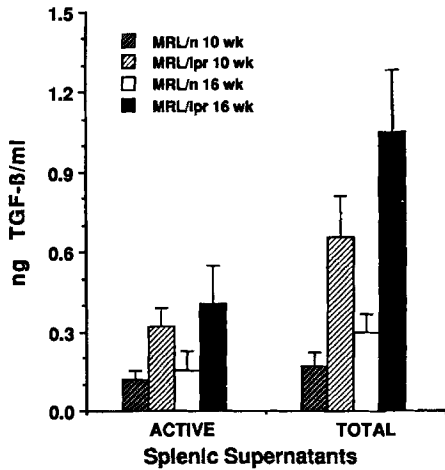
Figure 5. Comparison of both active and total TGF-β levels in 24-h resting spleen cell cultures from male MRL/n ($n = 3$) and MRL/lpr mice ($n = 3$). TGF-β was measured by bioassay with mink lung cells. MRL/lpr mice have significantly higher levels of both active ($p < 0.05$) and total TGF-β ($p < 0.05$, Mann-Whitney U test) at both 10 and 16 wk of age.

We also investigated the levels of active TGF-β present in the peritoneal exudates of MRL/n and MRL/lpr mice 4, 24, and 48 h after intraperitoneal SA1 challenge. At 4 h, TGF-β was not detectable in the peritoneal exudates of either strain. However, at 24 h MRL/lpr mice had greater concentrations of active TGF-β in the peritoneum than did MRL/n mice but these levels were not significantly different (Fig. 6). In contrast, at 48 h the levels of active TGF-β in MRL/lpr exudates increased significantly over those of MRL/n mice ($p < 0.03$), which were identical to the concentrations observed at 24 h (Fig. 6). These results demonstrate that MRL/lpr mice had elevated levels of active TGF-β at the site of infection and that these levels reach significance after the increase in bacterial burden and increase in PMN number. In addition, they suggest that spontaneous production of active TGF-β may contribute to the increased risk of infection observed in MRL/lpr mice.

Intravenous Injection of Active TGF-β1 into MRL/n Mice Mimics the Host Defense Defect Observed in MRL/lpr Mice. To

Figure 6. Comparison of active TGF-β levels per ml of peritoneal lavage from MRL/n and MRL/lpr mice 24 and 48 h after intraperitoneal challenge with 6×10^4 CFU of SA1. At 24 h, levels of active TGF-β are not significantly different between MRL/n ($n = 7$) and MRL/lpr mice ($n = 6$). However, at 48 h the levels are significantly different between MRL/n ($n = 9$) and MRL/lpr mice ($n = 8$) ($p < 0.03$, Mann-Whitney U test).

link the elevated levels of active TGF-β we observed in MRL/lpr splenic supernatants with the increased risk of bacterial infection observed in these mice, we injected MRL/n mice parenterally with a single dose of active TGF-β1 to determine whether we could mimic the response of MRL/lpr mice to bacterial challenge with SA1. If TGF-β had an effect on host susceptibility to bacterial infection in MRL/lpr mice, then treating MRL/n mice with TGF-β1 should reproduce the responses observed in MRL/lpr mice. Therefore we injected 11-wk-old male MRL/n mice with a single intravenous dose of either vehicle control or activated TGF-β1 immediately before intraperitoneal challenge with 8.5×10^4 CFU of SA1. At 4 h after bacterial challenge, vehicle control-injected MRL/n mice had a significantly greater percentage and number of PMN in the peritoneal lavage than did the TGF-β-injected MRL/n mice ($p < 0.02$ and $p < 0.02$, respectively) (Fig. 7, A and B). A decrease in the number and per-
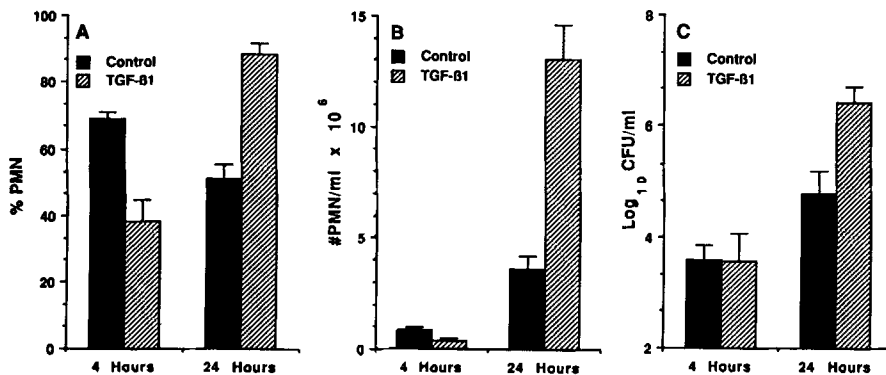
Figure 7. Comparison of MRL/n injected via the tail vein with either 300 ng of active TGF-β1 or vehicle control on the (A) percent PMN, (B) PMN number, and (C) $\log_{10}$ CFU/ml of a lavage of the peritoneal cavity 4 and 24 h after intraperitoneal challenge with 8.5×10^4 CFU of SA1. At 4 h, the percent PMN and PMN number/ml, but not the CFU/ml, were significantly different between TGF-β1-injected ($n = 4$) and vehicle control-injected mice ($n = 5$) ($p < 0.02$ and $p < 0.02$, respectively, Mann-Whitney U test). At 24 h, the percent PMN, PMN number/ml, and the CFU/ml were significantly different between TGF-β1-treated ($n = 13$) and control-treated mice ($n = 11$) ($p < 0.0002$, $p < 0.0002$, and $p < 0.004$, respectively, Mann-Whitney U test).

A single injection of active TGF-β1 into MRL/n mice at the time of bacterial challenge duplicates both the alterations in PMN extravasation and the increased bacterial burden observed in MRL/lpr mice within the first 24 h of bacterial infection.

centage of PMN present in the peritoneal exudates from TGF-β1–treated MRL/n mice at 4 h after bacterial challenge mimics the response of MRL/lpr mice at the same time point. No differences between the two strains in the recovery of viable bacteria from the peritoneal lavage were observed at this early time point (Fig. 7 C).

At 24 h after bacterial challenge, both the percentage and number of PMN present in the peritoneal exudate were significantly higher in TGF-β1–treated mice, mimicking the PMN response observed in MRL/lpr mice at 24 h after challenge ($p < 0.0002$ and $p < 0.0002$, respectively) (Fig. 7, A and B). TGF-β1–treated MRL/n mice also had significantly more bacteria in the peritoneal lavage than did the vehicle control-treated MRL/n mice ($p < 0.004$) (Fig. 7 C). In addition, TGF-β1–treated mice also had significantly higher numbers of bacteria in the liver than did control-treated mice (data not shown). Comparison of bacterial burden in the peritoneal lavage 4 and 24 h after intraperitoneal challenge shows that the TGF-β1–injected animals were unable to restrict the growth of the microorganism over this 20-h period as compared with control-treated mice (Fig. 7 C). These observations demonstrate that a single intravenous injection of active TGF-β1 into MRL/n mice at the time of bacterial challenge can duplicate both the alterations in PMN extravasation and the increased bacterial burden observed in MRL/lpr mice within the first 24 h after intraperitoneal challenge with SA1.

Injection of Anti-TGF-β1 into MRL/lpr Mice Significantly Ameliorates Their Defect in Host Defense against Bacterial Challenge. To further confirm our hypothesis that altered regulation of TGF-β in MRL/lpr mice is affecting host response to bacterial infection, we assessed the effect of a single dose of mAb against TGF-β injected intravenously into MRL/lpr mice at the time of bacterial challenge. MRL/lpr mice were injected intravenously with 166 μg of either purified monoclonal anti-TGF-β or purified murine IgG1 as a control. Immediately after the injection of the anti-TGF-β or control IgG, the mice were injected intraperitoneally with $2.5-5 \times 10^4$ CFU of SA1. 4 h after the bacterial challenge, the percent and number of PMN in the peritoneal lavage were significantly

elevated in the anti-TGF-β–treated mice as compared with those injected with control IgG1 ($p < 0.05$, for each, Fig. 8, A and B). At this time the bacterial burden in the peritoneal lavage was not significantly different (Fig. 8 C). 24 h after the bacterial challenge, the percent and number of PMN in the peritoneal lavage were significantly lower in the anti-TGF-β–treated mice ($p < 0.003$ and $p < 0.001$, respectively) (Fig. 8, A and B). The percent and number of PMN present in the anti-TGF-β–treated MRL/lpr mice were similar to the values observed in MRL/n mice for both 4 and 24 h after intraperitoneal challenge with SA1 (Fig. 4, A and B). Importantly, the anti-TGF-β–treated mice had significantly fewer bacteria in the peritoneal lavage as compared with the control-treated MRL/lpr mice ($p < 0.006$) (Fig. 8 C). In fact, treatment with anti-TGF-β reduced the CFU in the peritoneal lavage from $6.05 \pm 2.7 \times 10^6$/ml to $0.44 \pm 0.13 \times 10^6$/ml, a reduction of 93%. Therefore, treatment of MRL/lpr mice with anti-TGF-β ameliorates their alteration in PMN extravasation into the peritoneum and decreases their resulting bacterial burden at that site.

To determine whether the ability of anti-TGF-β to reduce the CFU in the peritoneal lavage correlated with survival of MRL/lpr mice in response to SA1 challenge, we assessed the survival of control IgG1- and anti-TGF-β–treated MRL/lpr mice after intraperitoneal challenge with 9×10^4 CFU of SA1. As shown in Fig. 9, the survival of MRL/lpr mice treated with 166 μg of anti-TGF-β was significantly increased over the survival of control-treated mice ($p < 0.02$). These data indicate that the reduction of CFU in the peritoneal lavage observed with anti-TGF-β treatment (Fig. 8 C) correlates with survival of MRL/lpr mice after bacterial challenge. In conclusion, these data show that the failure of MRL/lpr mice to clear a bacterial infection and to contain the infection to a local site is due, at least in part, to the elaboration of active TGF-β.

Discussion

We show in this paper that spontaneous elaboration of active TGF-β adversely affects host defense against both gram-
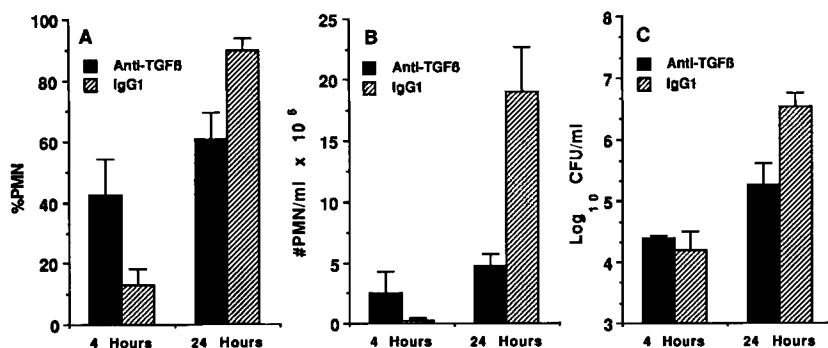
Figure 8. Comparison of MRL/lpr mice injected via the tail vein with either 166 μg of monoclonal murine IgG1 anti-TGF-β (1D11.16) or control murine IgG1 on the (A) percent PMN, (B) PMN number, and (C) log$_{10}$ CFU/ml of a lavage of the peritoneal cavity 4 and 24 h after intraperitoneal challenge with $2.4-5.0 \times 10^4$ CFU of SA1. At 4 h the percent PMN and PMN number/ml, but not the CFU/ml, were significantly different between anti-TGF-β–treated ($n = 4$) and control-treated mice ($n = 4$) ($p < 0.05$ for each, Mann-Whitney U test). At 24 h, the percent PMN, PMN number/ml, and the CFU/ml were significantly different between anti-TGF-β–treated ($n = 13$) and control-treated mice ($n = 12$) ($p < 0.003$, $p < 0.001$, and $p < 0.006$, respectively, Mann-Whitney U test). Treatment of MRL/lpr mice with anti-TGF-β ameliorates their alteration in PMN extravasation into the peritoneum and decreases their resulting bacterial burden at that site.
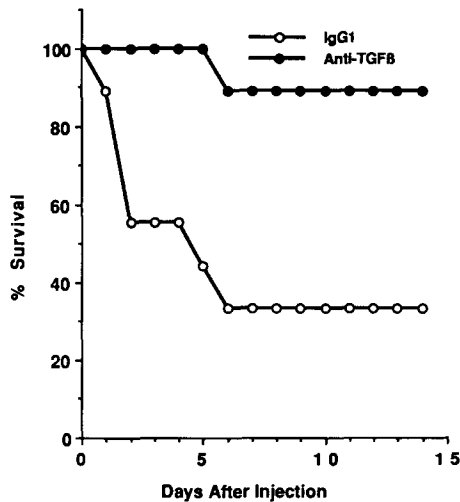
Figure 9. Effect of *S. aureus* SA1 injected intraperitoneal on the survival of 12–13-wk-old male MLR/lpr mice injected via the tail vein with either 166 μg of monoclonal murine IgG1 anti-TGF-β (1D11.16) (*n* = 9) or control murine IgG1 (*n* = 9). Animals were challenged intraperitoneal with 9.0 × 10⁴ CFU of SA1. Survival is significantly increased for male MRL/lpr mice treated with anti-TGF-β (*p* <0.02, Fisher's exact test).

negative and gram-positive bacterial infection in MRL/lpr mice, a murine model of systemic autoimmune disease. We derive this conclusion from the following data: (*a*) MRL/lpr mice, as compared with congenic MRL/n mice, exhibit elevated levels of active TGF-β at the site of infection (Fig. 6) and in supernatants of resting spleen cell cultures (Fig. 5). (*b*) PMN from MRL/lpr mice, but not MRL/n mice, fail to migrate to the site of infection during the initial stages of infection (Fig. 3). (*c*) MRL/lpr mice have a significantly increased bacterial burden at the site of infection and at other tissue sites 24 h after bacterial challenge as compared with MRL/n mice. Moreover, this increased bacterial burden occurs even though the percentage and number of PMN present at the site of infection are increased in MRL/lpr mice (Figs. 3 and 4). (*d*) The alteration in PMN extravasation during the initial stages of infection and the failure to restrict bacterial growth in vivo could be duplicated in MRL/n mice with a parenteral injection of active TGF-β1 at the time of bacterial challenge (Fig. 7). (*e*) The altercations in host defense and decreased survival in response to infection in MRL/lpr mice could be ameliorated by the parenteral administration of a mAb that neutralizes the activity of TGF-β (Figs. 8 and 9). Thus, we believe that TGF-β induces a defect in host defense in MRL/lpr mice that contributes to their decreased survival in response to either gram-positive or gram-negative bacterial infection (Figs. 1, 2, and 9).

These data extend our previous work where we examined PMN function in MRL/lpr and MRL/n mice (11). In that work, we showed that MRL/lpr PMN had an acquired defect in amplification of Fc receptor–mediated phagocytosis and in extravasation into a site of inflammation (thioglycollate-inflamed peritoneum). Incubation of normal murine PMN in serum from MRL/lpr mice induced these defects and treatment of MRL/lpr serum with anti-TGF-β abrogated the in-

duction of the PMN defects. In addition, incubation of normal PMN with purified TGF-β1 induced identical defects in PMN function. Moreover, both MRL/lpr PMN and TGF-β1–treated MRL/n PMN regained normal function after incubation in tissue culture media for 1–2 h in the absence of TGF-β (Gresham, H., and F. O'Sullivan, unpublished observation). These data indicated that the novel defect in PMN function in MRL/lpr mice was acquired and rapidly reversible and therefore, might be ameliorated by therapeutic intervention.

TGF-β is a potent immunosuppressive cytokine that has been implicated in the susceptibility to infection with intracellular pathogens such as *Trypanosoma cruzi* and *Leishmania* (21, 20). Macrophages infected with these microorganisms produce active TGF-β, which in turn suppresses normal cytokine-stimulated macrophage cytotoxic activity and allows for the permissive growth of these pathogens. Our study extends these observations to include a role for TGF-β in the regulation of events within 24 h after infection with encapsulated extracellular bacterial pathogens. Interestingly, these studies, like our own, demonstrate that active TGF-β given at the time of infection can convert nonsusceptible murine strains into permissive environments for the growth of the microorganism and that giving anti-TGF-β at the time of infection provides protection to susceptible murine strains. In fact, when we gave either TGF-β or anti-TGF-β 24 h before infection, there was no effect on the infectious process in the appropriate murine strain (Lowrance, J., and H. Gresham, unpublished observation). In total, these studies suggest that active TGF-β is involved in early events during infection with both intracellular parasites and extracellular bacteria.

Encapsulated bacteria like *E. coli* and *S. aureus* use distinctly different mechanisms for invasion in vivo and for evasion of host defenses than do intracellular organisms like *T. cruzi* and *Leishmania*. Therefore, the targets for the effects of TGF-β are likely to be different in these two types of infectious processes. In this regard, we believe that TGF-β is adversely affecting PMN function at two separate sites; once early in the infection by retarding PMN extravasation from the vasculature and later by inhibiting normal bacteriostatic activity at the site of the infection. The failure of MRL/lpr PMN to extravasate into the peritoneum could be explained by an effect of TGF-β on the endothelium because TGF-β treatment of endothelium reduces the adhesiveness of human PMN for the endothelial surface (32). In addition, disruption of the TGF-β1 gene creates TGF-β1–deficient mice that die from an overwhelming multifocal inflammatory disease due to the unregulated infiltration of leukocytes into various tissues (33, 34). These studies suggest that TGF-β1 plays an important role in regulating leukocyte movement across the endothelium. Alternatively, exposure to TGF-β1 in the vasculature may reduce the responsiveness of PMN to chemotactic stimuli generated at the site of infection. In this regard, intravenous administration of TGF-β1 suppresses leukocyte recruitment to the inflamed synovium in SCW-induced arthritis in rats and anti-TGF-β has a similar effect when injected intraarticularly (18, 35). Because TGF-β1 is chemotactic for PMN, monocytes, and lymphocytes (36–39), one interpretation of

these data is that these treatments disrupt the chemotactic gradient of TGF-β1 established across the endothelium. Because we could see effects of TGF-β1 on PMN extravasation within 1–2 h and much longer incubation times are required for the inhibitory effects of TGF-β1 on endothelial-PMN adhesion (32), we are currently favoring the latter hypothesis to explain our results.

The mechanism by which TGF-β inhibits host defense at the site of infection cannot be explained currently. Because our previous data demonstrated TGF-β–induced defects in PMN phagocytic function, one possibility to explain the decreased clearance of the pathogen from sites of infection is decreased phagocytic uptake of the organisms by PMN. However, these organisms are not readily ingested by either normal PMN or macrophages, consistent with the presence of the capsule (Gresham, H., unpublished observation). In addition, neither normal PMN nor macrophages have bactericidal activity in vitro for the encapsulated bacteria used in our studies, even in the presence of complement (Lowrance, J., and H. Gresham, unpublished observation). Therefore, we believe that the effect of TGF-β we observed on host defense is mediated by the suppression of bacteriostatic activity in vivo (Fig. 4 C). This is in contrast to the effect of TGF-β on the cytotoxic activity of macrophages against intracellular pathogens (20, 21). Thus, TGF-β may adversely affect both bacteriostatic and bactericidal host defense mechanisms. The precise bacteriostatic mechanisms inhibited by TGF-β remain to be elucidated. Efforts are underway to develop an in vitro test of PMN function in which we can assess bacteriostatic parameters.

How our data relate to what is known about infection and PMN function in humans is also unclear. It's intriguing to speculate that TGF-β–mediated suppression of PMN function underlies the risk of bacterial infection and the defective PMN function observed in several human diseases, including diabetes mellitus (40, 41), AIDS (42, 43), and autoimmune diseases like SLE and rheumatoid arthritis (RA) (1–7, 44–46). Infections with common extracellular bacterial pathogens are significant causes of morbidity and mortality in all of these diseases (1–4, 40, 42) and defects in PMN function similar to those we have observed in vitro and in vivo in MRL/lpr mice (11, and this paper) have been observed in patients with RA (44, 45), SLE (5–7), diabetes mellitus (41), and AIDS (43). Moreover, elevated TGF-β has been detected, either systemically or at the site of inflammation, in each of these diseases. TGF-β is elevated in the synovial fluid from patients with RA (46) and in the plasma of AIDS patients (22, 23, 47). Cultured mononuclear cells from patients with SLE produce increased levels of active TGF-β as compared with normal controls (24), and TGF-β is present in the glomerulus of kidneys from patients with type I diabetic nephropathy (48). Therefore, we believe that MRL/lpr mice may represent a good model for elucidation of these host defense defects and for assessment of agents which augment host defense against bacterial infection.

Studies are currently underway in our laboratories to determine the cellular source of the active TGF-β produced in these mice and to determine the relationship of this TGF-β production to the autoimmune disease observed in MRL/lpr mice. We suspect that the elaboration of active TGF-β during the course of an autoimmune disease may be a homeostatic mechanism for suppression of exaggerated and inappropriate immunostimulation. Evidence for this comes from the fact that TGF-β has been implicated in the spontaneous resolution of murine EAE (19), that TGF-β1 suppresses autoantibody levels in several murine models of autoimmunity, including MRL/lpr (15–18, 49), and that treatment of these animals with anti-TGF-β increases their concentration of autoantibodies (15, 16). Thus, some of the pathological sequelae observed in autoimmune diseases may develop as a consequence of this elaboration of TGF-β. In this regard, TGF-β–mediated matrix deposition in the glomerulus appears to play a significant role in decreased renal function observed in rat models of autoimmune glomerulonephritis (48, 50) and in human type I diabetic nephropathy (48). Therefore, we propose that an increased risk of infection is also a consequence of altered TGF-β expression. It is intriguing to speculate that the use of TGF-β antagonists may represent a rational approach for augmenting host defense against life-threatening infections in patients with autoimmune diseases.

References

1. Reveille, J.D., A. Bartolucci, and G.S. Alarcon. 1990. Prognosis in systemic lupus erythematosus. Negative impact of increasing age at onset, black race, and thrombocytopenia, as

   well as causes of death. *Arthritis Rheum.* 33:37.
2. Duffy, K.N.W., C.M. Duffy, and D.D. Gladman. 1991. Infection and disease activity in systemic lupus erythematosus: a

review of hospitalized patients. *J. Rheumatol.* 18:1180.

3. Mitchell, S.R., P.Q. Nguyen, and P. Katz. 1990. Increased risk of Neisserial infections in systemic lupus erythematosus. *Semin. Arthritis. Rheum.* 20:174.

4. Rosner, S., E. Ginzler, H. Diamond, M. Weiner, M. Schlessinger, J.F. Fries, C. Wasner, T.A. Medsger, 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.

5. Brandt, L., and H. Hedberg. 1969. Impaired phagocytosis by peripheral blood granulocytes in systemic lupus erythematosus. *Scand. J. Haematol.* 6:348.

6. Goetzl, E. 1976. Defective responsiveness to ascorbic acid of neutrophil random and chemotactic migration in Felty's syndrome and systemic plus erythematosus. *Ann. Rheum. Dis.* 33:167.

7. Clark, R., H. Kimball, and J. Decker. 1974. Neutrophil chemotaxis in systemic lupus erythematosus. *Ann. Rheum. Dis.* 33:167.

8. Theofilopoulos, A.N., and F.J. Dixon. 1985. Murine models of systemic lupus erythematosus. *Adv. Immunol.* 37:269.

9. Cohen, P.L., and R.A. Eisenberg. 1991. Lpr and gld: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol.* 9:243.

10. O'Sullivan, F.X., C.J. Ray, Y. Takeda, G.C. Sharp, and S.E. Walker. 1991. Long-term anti-CD4 treatment of MRL/lpr mice ameliorates immunopathology and lymphoproliferation but fails to suppress rheumatoid factor production. *Clin. Immunol. Immunopathol.* 61:421.

11. 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.

12. Roberts, A.B., and M.B. Sporn. 1990. The transforming growth factor-βs. *Handb. Exp. Pharmacol.* 95:419.

13. Wahl, S.M. 1992. Transforming growth factor beta (TGF-β) in inflammation: a cause and a cure. *J. Clin. Immunol.* 12:61.

14. Border, W.A., and E. Ruoslahti. 1992. Transforming growth factor-β in disease: the dark side of tissue repair. *J. Clin. Invest.* 90:1.

15. Kuruvilla, A.P., R. Shah, G.M. Hochwald, H.D. Ligitt, 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.

16. Racke, M.K., S. Dhib-Jalbut, B. Cannella, P.S. Albert, C. Raine, and D.E. McFarlin. 1991. Prevention and treatment of chronic relapsing allergic encephalomyelitis by transforming growth factor β1. *J. Immunol.* 146:3012.

17. Johns, L.D., K.C. Flanders, G.E. Ranges, and S. Sriram. 1991. Successful treatment of experimental allergic encephalomyelitis with transforming growth factor β1. *J. Immunol.* 147:1792.

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.

19. Khoury, S.I., W.W. Hancock, and H.L. Weiner. 1992. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor β, interleukin 4, and prostaglandin E expression in the brain. *J. Exp. Med.* 176:1355.

20. Barral-Netto, M., A. Barral, C. Brownell, Y.A.W. Skeiky, L.R. Ellingsworth, D.R. Twardzik, and S.G. Reed. 1992. Transforming growth factor-β in Leishmanial infection: a parasite escape mechanism. *Science (Wash. DC).* 257:545.

21. Silva, J.S., D.R. Twardzik, and S.G. Reed. 1991. Regulation of *Trypanosoma cruzi* infections in vitro and in vivo by transforming growth factor β (TGF-β). *J. Exp. Med.* 174:539.

22. Kekow, J., W. Wachsman, J.A. McCutchan, M. Cronin, D.A. Carson, and M. Lotz. 1990. Transforming growth factor-β and noncytopathic mechanisms of immunodeficiency in human immunodeficiency virus infection. *Proc. Natl. Acad. Sci. USA.* 87:8321.

23. Kekow, J., W. Wachsman, J.A. McCutchan, W.L. Cross, M. Zachariah, D.A. Carson, and M. Lotz M. 1991. Transforming growth factor-β and suppression of humoral immune responses in HIV infection. *J. Clin. Invest.* 87:1010.

24. DelGiudice, G., K. Elkon, and M. Crow. 1993. TGF-β activity is increased in systemic lupus erythematosus (SLE) and progressive systemic sclerosis (PSS). *Arthritis Rheum.* 36:S196. (Abstr.)

25. Cross, A.S., J.C. Sadoff, N. Kelly, E. Bernton, and P. Gemski. 1989. Pretreatment with recombinant murine tumor necrosis factor α/cachectin and murine interleukin 1 α protects mice from lethal bacterial infection. *J. Exp. Med.* 169:2021.

26. Lee, J.C., M.J. Betley, C.A. Hopkins, N.E. Perez, and G.B. Pier. 1987. Virulence studies, in mice, of transposon-induced mutants of Staphylococcus aureus differing in capsule size. *J. Infect. Dis.* 156:741.

27. Baddour, L.M., C. Lowrance, A. Albus, J.H. Lowrance, S.K. Anderson, and J.C. Lee. 1992. *Staphylococcus aureus* microcapsule expression attenuates bacterial virulence in a rat model of experimental endocarditis. *J. Infect. Dis.* 165:749.

28. Merino, J., J.A. Casado, J. Cid, A. Sanchez-Ibarrola, and S. Subir. 1992. The measurement of transforming growth factor-β levels produced by peripheral blood mononuclear cells requires the efficient elimination of contaminating platelets. *J. Immunol. Methods.* 153:151.

29. Danielpour, D., K.L. Dart, K.C. Flanders, A.B. Roberts, and M.B. Sporn. 1989. Immunodetection and quantitation of the two forms of transforming growth factor-β (TGF-β1 and TGF-β2) secreted by cells in culture. *J. Cell Physiol.* 138:79.

30. 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.

31. Curnutte, J.C. 1988. Chronic granulomatous disease: clinical and genetic aspects. *In* R.I. Lehrer, moderator. Neutrophils and Host Defense. *Ann. Int. Med.* 109:127.

32. Gamble, J.R., and M.A. Vadas. 1988. Endothelial adhesiveness for blood neutrophils is inhibited by transforming growth factor-β. *Science (Wash. DC).* 242:97.

33. Shull, M.M., I. Ormsby, A.B. Kier, S. Pawlowski, R.J. Diebold, Z.M. Yin, R. Allen, C. Sidman, G. Proetzel, D. Calvin, N. Annunziata, and T. Doetschman. 1992. Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature (Lond.).* 359:693.

34. Kulkarni, A.B., C. Huh, D. Becker, A. Geiser, M. Lyght, K.C. Flanders, A.B. Roberts, M.B. Sporn, J.M. Ward, and S. Karlsson. 1993. Transforming growth factor-β1 null mutation in mice causes excessive inflammatory response and early death. *Proc. Natl. Acad. Sci. USA.* 90:770.

35. Wahl, S.M., J.B. Allen, G.L. Costa, H.L. Wong, and J.R. Dasch. 1993. Reversal of acute and chronic synovial inflam-

mation by anti-transforming growth factor β. *J. Exp. Med.* 177:225.

36. Brandes, M.E., U.E. Mai, K. Ohura, and S.M. Wahl. 1991. Human neutrophils express type I TGF-β receptors and chemotax to TGF-β. *J. Immunol.* 147:1600.

37. Reibman, J., S. Meixler, T.C. Lee, L.I. Gold, B.N. Cronstein, K.A. Haines, S.L. Kolasinski, and G. Weissman. 1991. Transforming growth factor β1, a potent chemattractant for human neutrophils, bypasses classic signal-transduction pathways. *Proc. Natl. Acad. Sci. USA.* 88:6805.

38. Wahl, S.M., D.A. Hunt, L. Wakefield, N. McCartney-Francis, L.M. Wahl, A.B. Roberts, and M.B. Sporn. 1987. Transforming growth factor-β induced monocyte chemotaxis and growth factor production. *Proc. Natl. Acad. Sci. USA.* 84:5788.

39. Adams, D.H., M. Hathaway, J. Shaw, D. Burnett, E. Elias, and A.J. Strain. 1991. Transforming growth factor-β induced human T lymphocyte migration in vitro. *J. Immunol.* 147:609.

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

41. Wilson, R.M., and W.G. Reeves. 1986. *Clin. Exp. Immunol.* 63:478.

42. 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.

43. Ellis, M., S. Gupta, S. Galant, S. Hakin, C. Van de Ven, C. Toy, and M.S. Cairo. 1988. Impaired neutrophil function in patients with AIDS or AIDS-related complex: a comprehen-

sive evaluation. *J. Infect. Dis.* 158:1268.

44. 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.

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

46. Fava, R., N. Olsen, J. Keski-Oja, H. Moses, and T. Pincus. 1989. Active and latent forms of transforming growth factor β activity in synovial effusions. *J. Exp. Med.* 169:291.

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

48. Yamamoto, T., T. Nakamura, N. Noble, E. Ruoslahti, and W. Border. 1993. Expression of transforming growth factor β is elevated in human and experimental diabetic nephropathy. *Proc. Natl. Acad. Sci. USA.* 90:1814.

49. 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. (Abstr.)

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