Autoimmunity Associated with TGF-β1-Deficiency in Mice Is Dependent on MHC Class II Antigen Expression

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Abstract

The progressive inflammatory process found in transforming growth factor β1 (TGF-β1)–deficient mice is associated with several manifestations of autoimmunity, including circulating antibodies to nuclear antigens, immune complex deposition, and increased expression of both class I and class II major histocompatibility complex (MHC) antigens. The contribution of MHC class II antigens to the genesis of this phenotype has been determined by crossing the TGF-β1-null [*TGF-β1$^{(-/-)}$*] genotype into the MHC class II–deficient [*MHC-II$^{(-/-)}$*] background. Mice homozygous for both the TGF-β1 null allele and the class II null allele [*TGF-β1$^{(-/-)}$;MHC-II$^{(-/-)}$*] are without evidence of inflammatory infiltrates, circulating autoantibodies, or glomerular immune complex deposits. Instead, these animals exhibit extensive extramedullary hematopoiesis with progressive splenomegaly and adenopathy, surviving only slightly longer than *TGF-β1$^{(-/-)}$;MHC-II$^{(+/+)}$* mice. The role of CD4^{+} T cells, which are also absent in MHC class II–deficient mice, is directly demonstrated through the administration of anti-CD4 monoclonal antibodies in class II–positive, *TGF-β1$^{(-/-)}$* mice. The observed reduction in inflammation and improved survival emphasize the significance of CD4^{+} cells in the pathogenesis of the autoimmune process and suggest that the additional absence of class II antigens in *TGF-β1$^{(-/-)}$;MHC-II$^{(-/-)}$* mice may contribute to their extreme myeloid metaplasia. Thus, MHC class II antigens are essential for the expression of autoimmunity in TGF-β1–deficient mice, and normally may cooperate with TGF-β1 to regulate hematopoiesis. (*J. Clin. Invest.* 1996. 98:2109–2119.) Key words: leukocyte • myeloid • hematopoiesis • inflammation • metaplasia

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Introduction

The immunoregulatory properties of transforming growth factor beta (TGF-β) are of critical importance to the maintenance of normal immunological homeostasis and cover a spectrum which extends from the regulation of adhesion molecule expression by endothelial cells and leukocytes (1–3), to the modulation of macrophage function (4, 5), and the control of lymphocyte activation and proliferation (6–9). Although each of the three mammalian TGF-β isoforms can modulate such activities when added exogenously to experimental systems, it is principally the type 1 isoform which appears to perform these functions in vivo (10–12). Given that it is the predominant and perhaps the only isoform produced by all classes of leukocytes, disruption of TGF-β1 gene expression should predictably result in profound alterations of normal immune function and should lead to distinct abnormalities in hematopoiesis. Indeed, targeted disruption of TGF-β1 gene expression in mice produces a phenotype which is characterized as a progressive multifocal inflammatory process (13, 14), associated with circulating autoantibodies and immune complex deposition (15), increased expression of both classes of MHC antigens (16), and also with dysregulated myelopoiesis as evidenced by increased numbers of circulating immature granulocytes, monocytes, and platelets (12).

Dissection of this complex phenotype has begun to identify some of the mechanisms which underlie the disease process resulting from TGF-β1-deficiency. Alterations in lymphocyte function and increased leukocyte–endothelial cell interactions have been implicated in the establishment of this inflammatory process (17, 18). The observed increased expression of the intercellular adhesion molecule-1 and the improved survival after the administration of antibodies to leukocyte function–associated antigen-1 suggest that alterations in the expression of cell surface adhesion molecules may initiate and promote tissue infiltration (19). Inherent abnormalities of leukocyte function are also implicated by the ability to transfer the phenotype via transplantation of *TGF-β1$^{(-/-)}$* bone marrow to lethally irradiated *TGF-β1$^{(+/+)}$* recipients (20). The central role of both B and T lymphocytes is substantiated further by the observation that the characteristic inflammatory lesions do not develop in TGF-β1–deficient mice crossed into the severe combined immunodeficiency (SCID)[1] background (19). We now directly demonstrate the potential for autoreactive T cells to marshal this abnormal immune response through the in vivo

1. *Abbreviation used in this paper:* SCID, severe combined immunodeficiency.

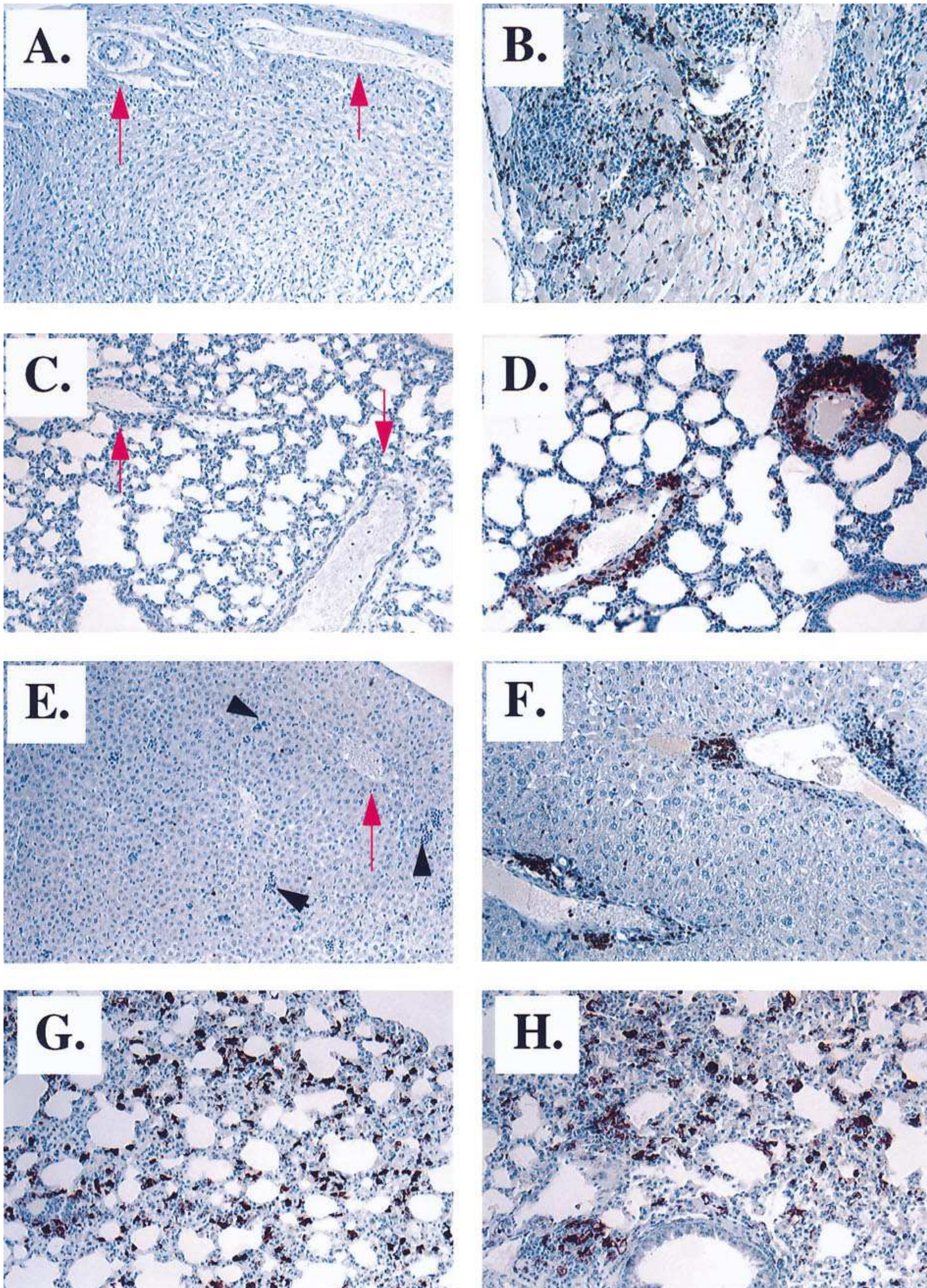

Figure 1. Development of mixed lymphocytic infiltrates in *TGF-β1*$^{(-/-)}$*;MHC*$^{(+/+)}$ but not *TGF-β1*$^{(-/-)}$*;MHC*$^{(-/-)}$ mice. Immunohistochemical staining of Bouin's-fixed tissue sections reveals characteristic perivascular accumulations of T lymphocytes positive for CD3 in *TGF-β1*$^{(-/-)}$*; MHC-II*$^{(+/+)}$ heart (*B*), lung (*D*), and liver (*F*). Analysis of these organs in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice (*A*, *C*, and *E*, respectively) demonstrates complete absence of infiltrates, with no discernible CD3 staining, except for a few resident T lymphocytes. Red arrows indicate longitudinal sections through blood vessels, illustrating the absence of perivascular accumulations. The accumulation of Mac-2–positive macrophages

depletion of CD4$^+$ T cells, with an attendant increase in survival and diminished inflammatory response in *TGF-β1*$^{(-/-)}$ mice.

The concept that the TGF-β1–deficient mouse represents a model of autoimmunity is based on the similarities which exist between the manifestations typical of autoimmune disease, such as circulating antibodies to nuclear antigens and immune complex deposition, and the abnormalities observed in *TGF-β1*$^{(-/-)}$ mice (10, 15). This concept is strengthened by data demonstrating the roles of endogenous TGF-β1 in modulating autoimmunity (21–23) and of exogenous TGF-β1 in both suppressing symptoms and preventing disease progression in various animal models of autoimmune disease (24, 25). In addition, the known associations between histocompatibility antigen expression and autoimmunity are reinforced by the observation that all *TGF-β1*$^{(-/-)}$ mice exhibit enhanced expression of both class I and class II MHC antigens at or before the onset of the inflammatory process (16, 26). The requirement for MHC class II molecules in the evolution of autoimmunity in TGF-β1–null mice was tested directly by crossing the *TGF-β1*$^{(-/-)}$ genotype onto an *MHC-II*$^{(-/-)}$ (CD4$^+$ T cell–deficient) background (27). The results suggest that the expression of autoimmunity in *TGF-β1*$^{(-/-)}$ mice is completely dependent on the presence of MHC class II antigen expression and accordingly requires the participation of the CD4$^+$ T cells with which these antigens interact. Finally, while MHC class II deficiency alone does not predispose to abnormalities in hematopoiesis, the presence of progressive extramedullary hematopoiesis in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice, predominantly a myeloid hyperplasia in the absence of inflammation, indicates that this aspect of the TGF-β1–deficient phenotype results directly from the loss of TGF-β1 function. Supporting this conclusion is our observation that enhanced myelopoiesis also exists in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(+/+)}$ mice in whom autoimmunity is impaired by the in vivo depletion of CD4$^+$ T cells. The potential significance of MHC class II–regulated interactions in this myeloproliferative process will also be discussed.

Methods

Mice. TGF-β1 heterozygous [*TGF-β1*$^{(+/-)}$] mice used in generating the following crosses were the offspring of matings between TGF-β1 heterozygotes established as previously described (14) and were of mixed C57BL/6J × 129/Sv genetic background. The MHC class II–deficient [*MHC-II*$^{(-/-)}$] mice (also of C57BL/6J × 129/Sv background) were of the H-2^b haplotype, so that disruption of the A^b_β gene resulted in the lack of cell surface expression of both I-E and I-A class II MHC molecules (27). *MHC-II*$^{(-/-)}$ mice were crossed to TGF-β1 heterozygotes to derive F_1 mice of the genotype *TGF-β1*$^{(+/-)}$*;MHC-II*$^{(+/-)}$. The latter were crossed to obtain F_2 mice that were of the *TGF-β1*$^{(+/-)}$*; MHC-II*$^{(-/-)}$ genotype, which served as founders to produce mice with the *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ genotype described in this study. Mice were maintained pathogen-free in a double-barrier facility, with autoclaved bedding and food.

DNA isolation and genotypic analysis. We determined the TGF-β1 genotypes of the offspring from each cross with previously described methods, using DNA isolated from a segment of distal tail (16). MHC class II genotypes for the F_1 and F_2 offspring were determined using a PCR method. Amplification primers for the normal allele were derived from exon 2 of the mouse I-A_β gene and give a product of 270 bp. The nucleotide sequences of these primers were 5′-GCATTTCGTGTACCAGTTCATGGG-3′ and 5′-AAGCCGCCGCAGGGAGGTGTGGG-3′. Primers for the mutant allele included the first of the above pair, with a second (sequence 5′-TGCGCTGACAGCCGGAACACGGCGG-3′) derived from within the neomycin resistance gene, giving a product of 600 bp. Once F_2 founders were established, determination only of TGF-β1 genotypes was required for offspring.

Histopathology and immunohistochemistry. Animals were killed by CO_2 narcosis and selected tissues were placed in either 10% neutral buffered formalin or Bouin's fixative, before paraffin embedding. Several 5-μm sections from each were stained with hematoxylin and eosin and analyzed. Bouin's-fixed tissue was used for immunohistochemical detection of staining for Mac-2 antigen with antibody obtained from Boehringer Mannheim (Indianapolis, IN), for B cells with the B220 antibody from Pharmingen (San Diego, CA), and for T cells with the CD3 antibody from Dako Corp. (Carpinteria, CA); Vectastain Elite ABC kits were obtained from Vector Laboratories Inc. (Burlingame, CA). Chloracetate esterase staining was performed on deparaffinized sections (4 μm) of formalin-fixed tissues using a diagnostic kit obtained from Sigma Chemical Co. (St. Louis, MO).

Glomerular Ig deposition. Kidney specimens were mounted in OCT, frozen on a bed of dry ice, and stored at −70°C. Cryostat sections 4 μm thick were cut and fixed in 10% acetone for 10 min. Alkaline phosphatase goat immunoconjugates to mouse IgG (Fc-specific or whole molecule) obtained from Sigma Chemical Co. were applied for 30 min. Slides were washed and exposed to Fast Red substrate from Dako Corp. to detect bound antibody, then counterstained with hematoxylin, all at room temperature.

Western blot analysis for antiribonucleoprotein antibodies. Mouse serum (1:150 dilution) was incubated with nitrocellulose strips containing nuclear extracts from Hep2 cells (ImmunoVision, Springdale, AR). The strips were processed and bound IgG was visualized as previously described (15), using goat anti–mouse IgG Ab from Bio-Rad (Hercules, CA).

Flow cytometric analysis. Single cell suspensions were prepared from spleen, lymph nodes, and bone marrow from at least four mice of each of the designated genotypes, and 1.5×10^6 cells were stained for expression of the designated lineage markers, with the following antibodies obtained from Pharmingen: anti–GR-1 and anti CD11b for myeloid lineages; anti-B220 for B cells, and anti-CD4, -CD5, and -CD8 for T cells; anti-CD18 (common β chain of the β2 integrin family, present on all mature leukocytes) was also used for these studies. Cells were incubated with primary antibodies and analyzed by flow cytometry.

Treatment with monoclonal antibody to L3T4. The rat IgG2b mAb to CD4 (GK1.5) and the control, nonimmune rat IgG (Ab49) were prepared in the Experimental Immunology Branch, NCI, NIH (28). A second mAb to L3T4 (H129.19) purchased from Boehringer Mannheim was also used in a separate set of in vivo depletion studies (29). A minimum of 10 *TGF-β1*$^{(-/-)}$ mice received intraperitoneal injections of anti-L3T4, starting with 1 mg on the first day of injections, and continuing with 0.5 mg (in 0.2 ml) on an every-other-day schedule, 3 d per week. Control groups included five *TGF-β1*$^{(+/+)}$ mice injected with mAb GK1.5 and five *TGF-β1*$^{(-/-)}$ mice that received Ab49. Animals were weighed each injection day. At time of killing, lymphocyte subpopulations in spleen and bone marrow were ana-

within lung is found in both the *MHC-II*$^{(+/+)}$ (*H*) and *MHC-II*$^{(-/-)}$ (*G*) backgrounds. The distribution is not in a focal, perivascular pattern as found for lymphocytes and may reflect increased numbers of circulating monocytes, which ultimately infiltrate into lung parenchyma and predominantly surround air spaces at the periphery of the lung. Arrowheads in *E* represent foci of extramedullary hematopoiesis which are primarily composed of granulocytes (see Fig. 7). *A*, *C*, and *E*, ×200; *B*, *D*, *F*, *G*, and *H*, ×400.

lyzed by flow cytometry, and tissues were submitted for evaluation by immunohistochemical studies (as above).

Results

Absence of inflammation in TGF-β1$^{(-/-)}$ mice lacking MHC class II expression. To determine if the development of inflammation in *TGF-β1*$^{(-/-)}$ mice is strictly dependent on histocompatibility antigen expression, we crossed *MHC-II*$^{(-/-)}$ mice with *TGF-β1*$^{(+/-)}$ mice to generate TGF-β1–deficient, MHC class II–null mice. Disruption of the TGF-β1 gene alone is associated with a marked increase in cell surface expression of both MHC class I and class II molecules, detected as early as day 4 after birth and perhaps serving as the signal to initiate tissue infiltration (16). Analysis of tissue sections from *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice revealed no evidence of the characteristic inflammatory lesions found in *TGF-β1*$^{(-/-)}$ mice, even after 21 d, when the process typically progresses rapidly and results in wasting and death (13, 14). Perivascular accumulation of T and B lymphocytes as identified by immunostaining for CD3 in *TGF-β1*$^{(-/-)}$ mice (Fig. 1, *B*, *D*, and *F*) is clearly absent in the *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice (Fig. 1, *A*, *C*, and *E*). Staining for B220 reveals a similar absence of infiltrating B lymphocytes in the *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice (not shown). The appearance of increased numbers of Mac-2–positive pulmonary macrophages and monocytes remains somewhat consistent between the two genotypes (Fig. 1, *G* and *H*) and may reflect the increased numbers of circulating monocytes in the peripheral blood (see below).

Reactivity towards nuclear antigens is absent from sera of TGF-β1$^{(-/-)}$;MHC-II$^{(-/-)}$ mice. Evidence for humoral autoimmunity in *TGF-β1*$^{(-/-)}$ mice includes the spontaneous production of immunoglobulins of the IgG isotype with antigen-binding specificities commonly seen both in human autoimmune conditions, such as systemic lupus erythematosus (30), and in animal models of autoimmune disease (31). These include specificities for dsDNA, ssDNA, and Sm ribonucleoprotein, with antigen-binding activity detectable in sera of *TGF-β1*$^{(-/-)}$ mice as early as 13 d of age (15). Western blot analysis for antiribonucleoprotein antibodies in *TGF-β1*$^{(-/-)}$*; MHC-II*$^{(-/-)}$ mice demonstrated complete loss of autoantibody production in the absence of class II antigen expression (Fig. 2), consistent with the lack of inflammatory lesions in these mice.

Immune complex deposition is not observed in TGF-β1$^{(-/-)}$; MHC$^{(-/-)}$ mice. Pathogenicity of circulating autoantibodies can be demonstrated by the formation of Ig complexes, which are often found as deposits in renal glomeruli. Such deposits are found in *TGF-β1*$^{(-/-)}$ mice as young as 12 d of age (15), but are never present in wild-type, age-matched siblings, and are distinctly absent when TGF-β1-deficiency co-exists with disrupted class II MHC antigen expression (Fig. 3). The absence of glomerular deposits correlates with the lack of circulating ribonucleoprotein-binding activity as shown in Fig. 2.

TGF-β1 deficiency in the absence of MHC class II antigen expression leads to myeloid metaplasia and decreased survival. Characterization of *TGF-β1*$^{(-/-)}$ mice demonstrated increased numbers of immature neutrophils, monocytes, and platelets circulating in the peripheral blood (13). Evidence of increased myelopoiesis is first evident in these mice as early as day 5 in the neonate, when a near twofold increase in myelopoietic cells is seen within the liver parenchyma (32). This enhanced myelopoiesis could potentially be driven by cyto-

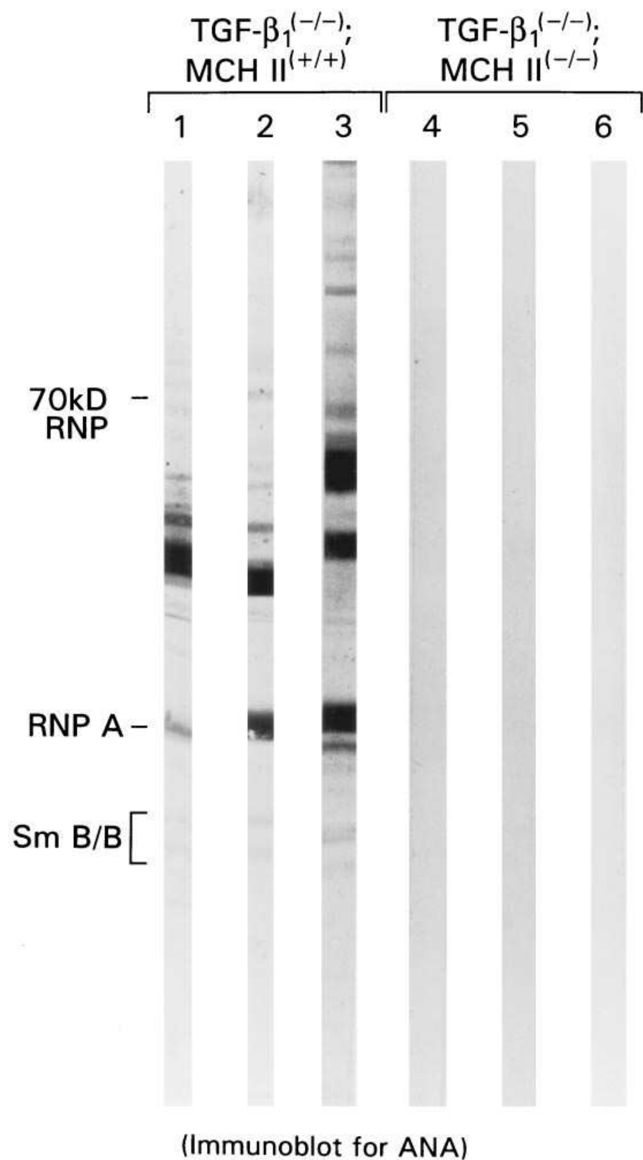

Figure 2. Antibodies to ribonucleoproteins are absent in *TGF-β1*$^{(-/-)}$*; MHC-II*$^{(-/-)}$ mice. Sera from *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(+/+)}$ (lanes *1–3*) and *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ (lanes *4–6*) mice were incubated with nitrocellulose strips containing nuclear extracts from Hep2 cells as described in Methods. The presence of antigen-binding activity in sera of *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(+/+)}$ mice can be detected as early as day 12 and displays heterogeneity typical of autoimmune reactions. The amount of bound antibody increases with age, but is never detected in the sera of the MHC-II–deficient, *TGF-β1*$^{(-/-)}$ mice (days 24–28 shown here).

kines released from cells participating in the systemic inflammatory process, but may also represent the loss of important inhibitory effects of TGF-β1 on this specific lineage of hematopoietic cells. We show here that, when compared with *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(+/+)}$ mice, *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice exhibit an accelerated myelopoiesis which is even more pronounced, leading to a dramatic increase in cellularity of the marrow compartment (Fig. 4, *B* and *C*). This relative marrow hyperplasia consists mainly of granulocytes, with significantly greater numbers of megakaryocytes, many appearing consid-

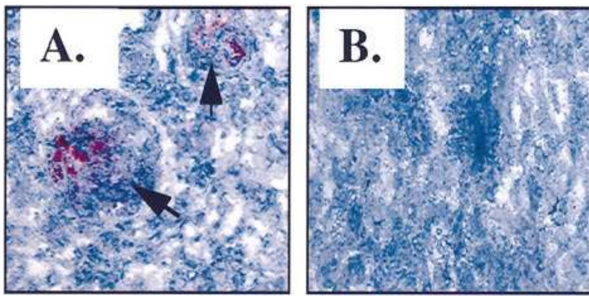

Figure 3. Glomerular Ig deposition is not a feature of the *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mouse. Cryostat sections of mouse kidneys stained with alkaline phosphatase immunoconjugates to mouse IgG reveal the presence of glomerular immune complex deposits in the kidneys of *TGF-β1*$^{(-/-}$*;MHC-II*$^{(+/+)}$ mice (*A*), but none were detected in kidneys of *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice (*B*). ×400.

erably larger in size (Fig. 4 *C*). Classically, myeloid metaplasia is a syndrome defined by the presence of anemia, enlargement of the spleen, circulating nucleated red blood cells and immature granulocytes, and conspicuous foci of extramedullary hematopoiesis (hematopoiesis occurring outside of the marrow space). Severe extramedullary hematopoiesis and myeloid hyperplasia in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice is shown by flow

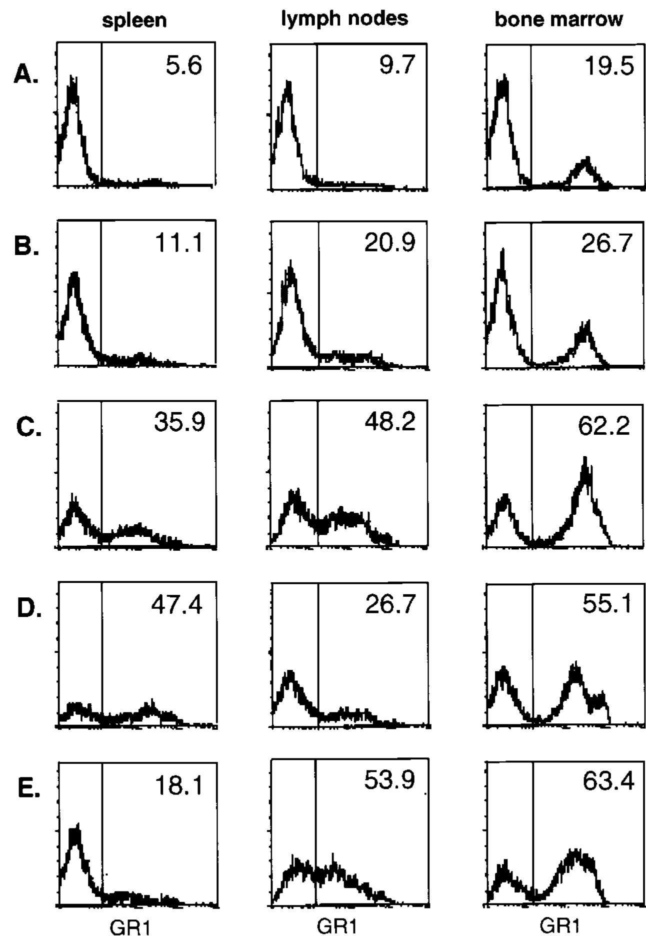

Figure 5. Extensive extramedullary hematopoiesis and myeloid metaplasia in *TGF-β1*$^{(-/-)}$ mice. Flow cytometry was performed on cells from spleen, lymph nodes, and bone marrow. Each row contains the results from a single animal, with percentages of GR1 positive cells indicated in the top right corner of each plot frame. A minimum of four animals of each genotype were analyzed, with representative individuals shown here. Wild-type mice at day 17 (*A*), compare with *TGF-β1*$^{(-/-)}$ mice at day 17 (*B*), and day 28 (*C*). *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ animals reveal a similar pattern as shown here at days 21 (*D*) and 26 (*E*). *TGF-β1*$^{(+/+)}$*;MHC-II*$^{(-/-)}$ mice resemble wild-type mice as in *A*.

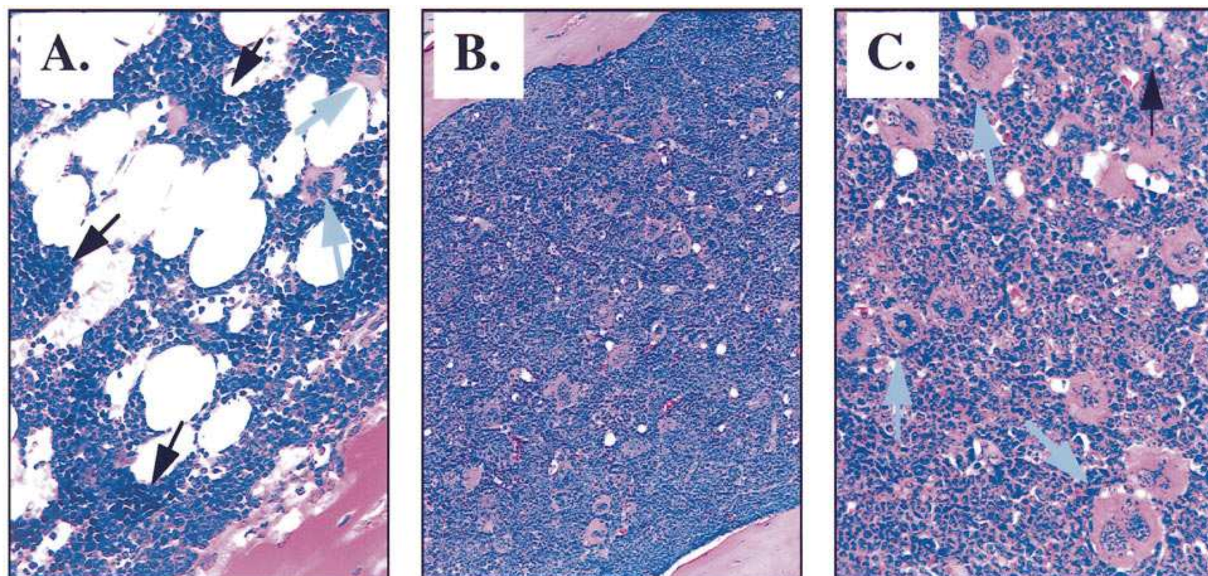

Figure 4. Increased cellularity of the bone marrow in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice, with increased numbers of myeloid elements. Note the normal cellularity with fat cells (white spaces) and foci of erythroid precursors (*black arrows*) present in wild-type animals (*A*); this is replaced by a hypercellular marrow (*B* and *C*), comprised of granulocytes and large numbers of megakaryocytes (*light blue arrows*). *C* contains at least 13 megakaryocytes in this single high-power field and many appear significantly larger than normal megakaryocytes as in *A*. A single mitotic megakaryocyte is indicated by the black arrow in *C*. Hematoxylin and eosin, *A* and *C*, ×400; *B*, ×200.

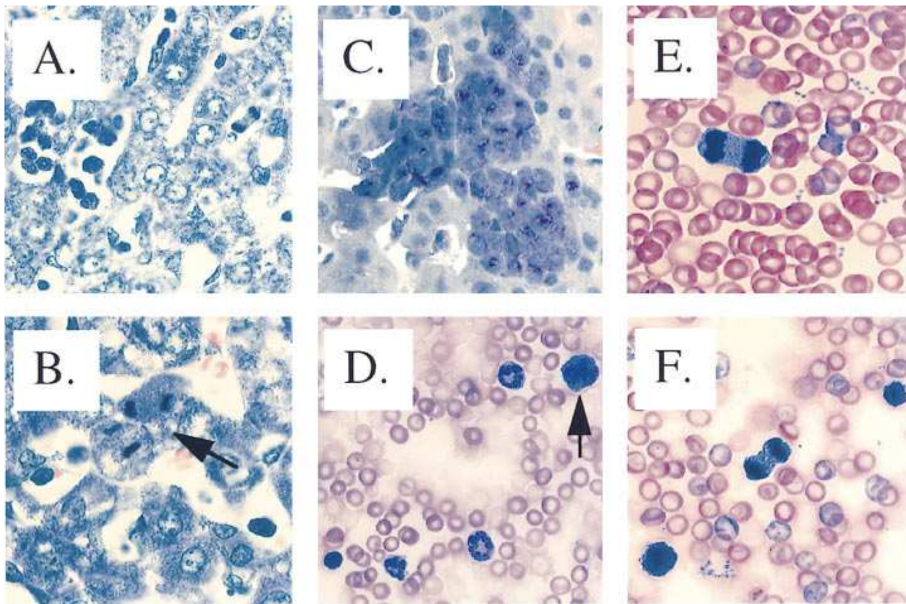

Figure 6. Extramedullary hematopoiesis associated with TGF-β1-deficiency is composed mainly of myeloid elements. Sections of $TGF\text{-}\beta 1^{(-/-)};MHC\text{-}II^{(-/-)}$ liver stained with Giemsa show numerous granulocytes within liver sinusoids (*A*), with the presence of mitotic figures in the larger progenitor cells (indicated by arrow in *B*) reflecting the active nature of the hematopoietic process in the liver. Staining for chloracetate esterase demonstrates that the majority of these cells are fully differentiated, designated by the presence of esterase (red) within mature granules (*C*). Giemsa-stained peripheral blood smears highlighting the severity of myeloid metaplasia (*D–F*); circulating erythroblasts (indicated by the arrow in *D* and shown relative to three granulocytes and a lymphocyte in the lower left corner), as well as the multiple mitotic figures evident within numerous circulating leukocytes (*E* and *F*), reflect the severity of anemia associated with the process (see Fig. 7). *A–F*, ×1,000.

cytometric analysis of their grossly enlarged lymph nodes and spleen. When compared with wild-type mice at day 17 (Fig. 5 *A*), $TGF\text{-}\beta 1^{(-/-)}$ mice consistently had greater numbers of myeloid cells (positive for GR1), with significant increases detected at day 17 (Fig. 5 *B*), progressing to fivefold this level by day 28 (Fig. 5 *C*) in $TGF\text{-}\beta 1^{(-/-)}$ lymph node and spleen, and threefold in bone marrow. $TGF\text{-}\beta 1^{(-/-)};MHC\text{-}II^{(-/-)}$ mice exhibit a very similar increase in GR1 positive cells, shown here at days 21 and 26 (Fig. 5, *D* and *E*). Islands of hematopoietic cells within the liver stain positively for chloracetate esterase, identifying them as mature granulocytes (Fig. 6 *C*), and active hematopoiesis is indicated by the larger, mitotic progenitors which are visualized within liver sinusoids (Fig. 6 *B*). The process is progressive, with splenomegaly and lymphadenopa-

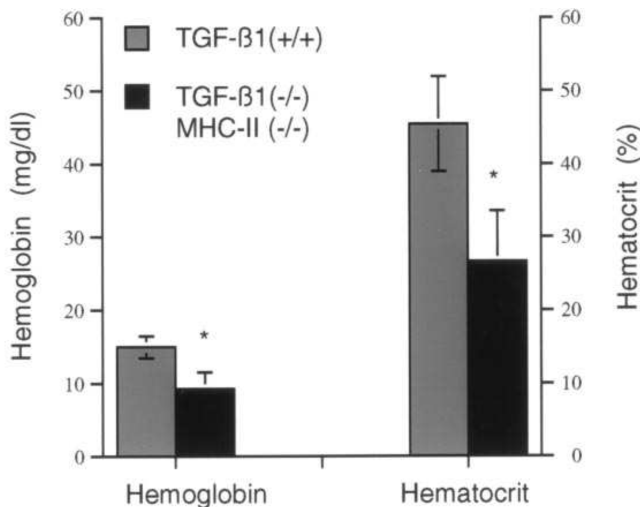

Figure 7. Anemia in $TGF\text{-}\beta 1^{(-/-)};MHC\text{-}II^{(-/-)}$ mice develops secondary to myeloid hyperplasia. Blood collected at the time of killing was analyzed for routine complete blood cell counts, including hemoglobin and hematocrit values. Data represent means of 10 $TGF\text{-}\beta 1^{(+/+)}$ and 13 $TGF\text{-}\beta 1^{(-/-)};MHC\text{-}II^{(-/-)}$ mice, all between 17 and 24 d of life. There is a significant reduction in the hemoglobin and hematocrit values in $TGF\text{-}\beta 1^{(-/-)};MHC\text{-}II^{(-/-)}$ mice ($^*P < 0.0001$). Results for wild-type are similar in either $MHC\text{-}II^{(+/+)}$ or $MHC\text{-}II^{(-/-)}$ backgrounds.

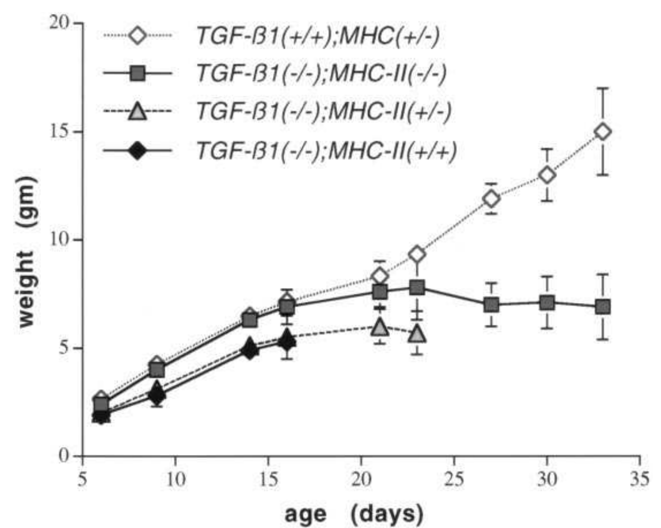

Figure 8. Growth and survival curves of $TGF\text{-}\beta 1^{(-/-)};MHC\text{-}II^{(-/-)}$ mice, with concurrent littermate $TGF\text{-}\beta 1^{(-/-)}$ controls [either wild-type (+/+) or heterozygous (+/−) for class II MHC antigens]. All TGF-β1-nulls represented here were the products of matings between $TGF\text{-}\beta 1^{(+/-)};MHC\text{-}II^{(+/-)}$ breeders, to allow for the direct, concurrent comparison of survival relative to class II expression. As demonstrated here, survival is minimally extended in $TGF\text{-}\beta 1^{(-/-)};MHC^{(-/-)}$ mice; each point represents the average weights on the designated day, and curves for all $TGF\text{-}\beta 1^{(-/-)}$ mice end at the average age at which animals were killed due to progressive symptoms (curves for TGF-β1 wild-type mice are only extended to the last point for $TGF\text{-}\beta 1^{(-/-)};MHC\text{-}II^{(-/-)}$, but their survival is otherwise normal).

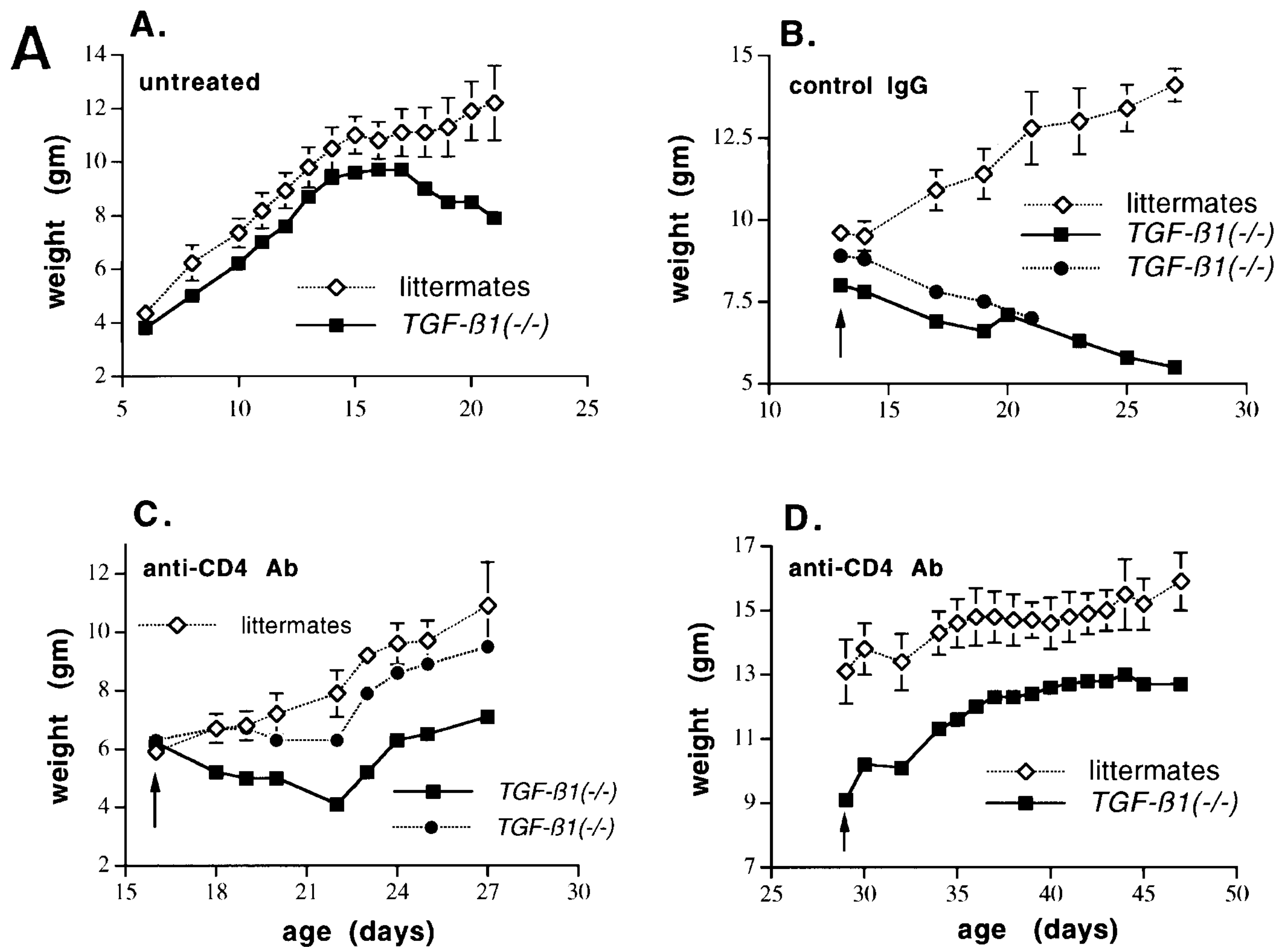

Figure 9. Anti-L3T4 monoclonal antibody treatment inhibits inflammation and improves survival of *TGF-β1*$^{(-/-)}$ mice. (*A*) Panel *A* demonstrates a typical growth curve for *TGF-β1*$^{(-/-)}$ mice, with weight loss beginning around day 15, and death by day 25, on average. Systemic administration of anti-CD4 antibodies results in increased survival and improved weight gain (panels *C* and *D*), while the administration of nonimmune IgG control does not (panel *B*). Even when antibody administration begins as late as day 28, symptoms improve and survival is extended (panel *D*; see also Fig. 10). Each panel represents a single litter, with curves of individual TGF-β1–null mice displayed with concurrent littermate controls. Arrows indicate the starting days for intraperitoneal injections. (*B*) Immunostaining for CD3$^+$ T cells demonstrates the presence of perivascular infiltrates in lung (panel *A*), liver (panel *C*), and heart (panel *E*), of *TGF-β1*$^{(-/-)}$ mice, which are clearly absent in the paired samples from anti-CD4 treated mice (panels *B*, *D*, and *F*; all tissues are from mice represented in *A*, panel *C*). (*C*) Flow cytometry demonstrates the absence of CD4$^+$ T cells in anti-L3T4 antibody–treated mice. To document that the observed effects are indeed the result of CD4 depletion, flow cytometry was performed to show that the CD4$^+$/CD5$^+$ T cell subset (demonstrated in wild-type, upper right corner of panel *A*) is absent in treated wild-type controls (*B*) and in *TGF-β1*$^{(-/-}$ mice (represented by *C* and *D*). Finally, there is a persistent increase in GR1$^+$ staining in spleen of CD4-depleted *TGF-β1*$^{(-/-)}$ mice (*F*), relative to wild-type control (*E*).

thy accompanied by the appearance of erythroblasts and mitotic leukocytes within the peripheral blood (Fig. 6, *D–F*). The presence of erythroid precursors is suggestive of an attempt to compensate for the anemia (Fig. 7), which may result from inefficient erythropoiesis in a crowded marrow compartment. While survival of *TGF-β1*$^{(-/-)}$;*MHC-II*$^{(-/-)}$ mice was generally longer than that of *TGF-β1*$^{(-/-)}$;*MHC-II*$^{(+/+)}$ or *TGF-β1*$^{(-/-)}$; *MHC-II*$^{(+/-)}$ mice in this colony, few of these double knockout mice survived beyond 5 wk (Fig. 8); this lethality is attributed to the progressive myeloid hyperplasia and its associated complications.

Injections of anti-L3T4 mAb effectively depletes CD4$^+$ *T cells in TGF-β1*$^{(-/-)}$ *mice, resulting in increased survival and a diminished inflammatory response.* Monoclonal antibodies to the murine T cell CD4 antigen have been used in mice to both prevent and reverse autoimmune disease in several murine models of human disorders (33–35). Treatment with anti-L3T4 (using either GK1.5 or H129.19) led to a significant increase in survival for *TGF-β1*$^{(-/-)}$ mice, along with a reduction in manifestations of autoimmunity, including an increase in weight gain (Fig. 9 *A*, panels *C* and *D*) and a decrease in perivascular inflammatory lesions (Fig. 9 *B*). Even when injections of mAb began beyond day 20 (Fig. 9 *A*, panel *D*; a period when symptoms typically are rapidly progressive), *TGF-β1*$^{(-/-)}$ mice responded with increased weight gain, improved appearance, and extended survival (as long as 80 d before time of killing; Fig. 10). Evaluation of lymphocyte subsets by flow cytometry demonstrated the effective depletion of CD4$^+$ T cell subset by

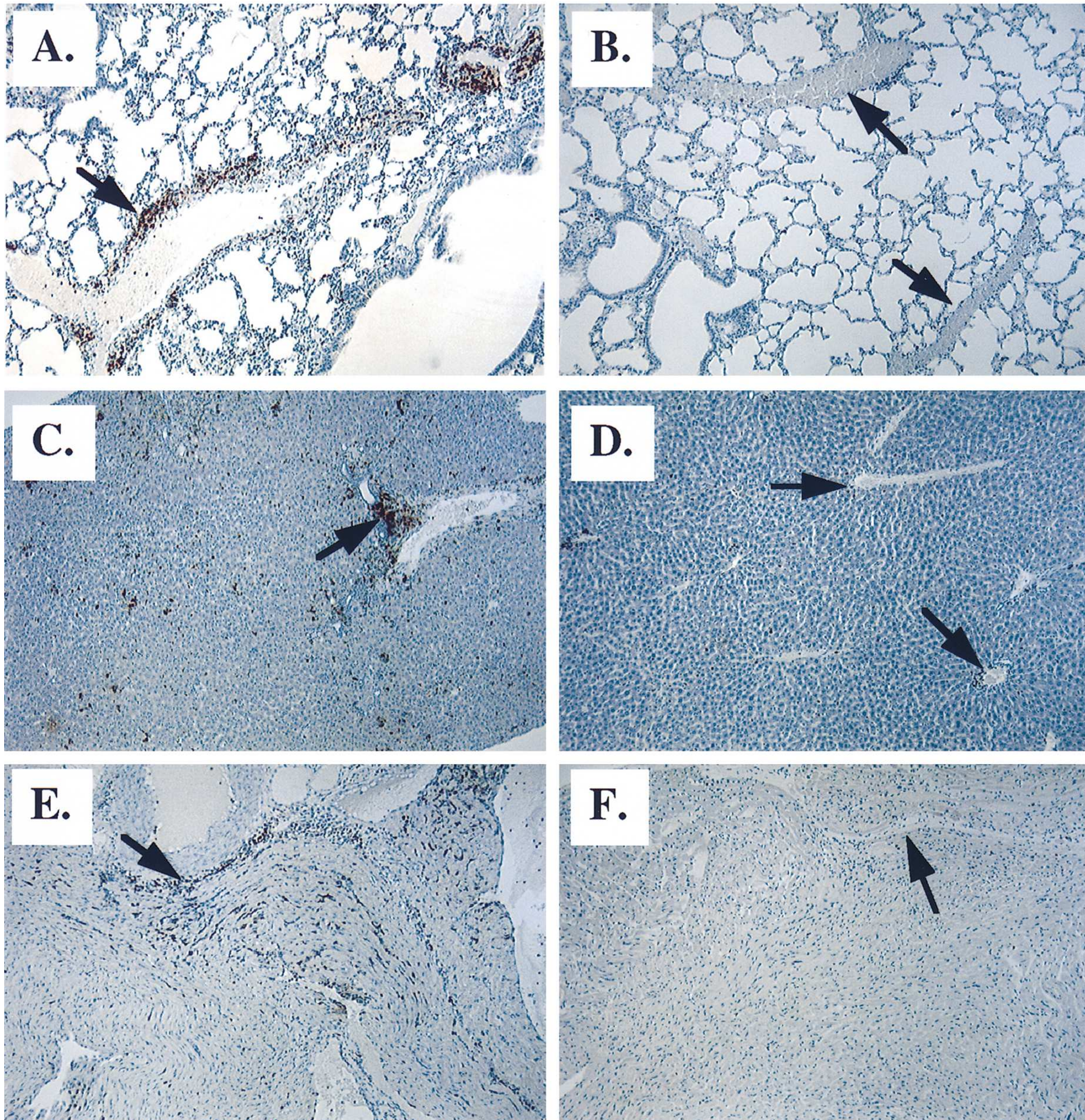

Figure 9.

mAb injections and reveals the persistent increase in GR1$^+$ cells similar to that observed in *TGF-β1*$^{(-/-)}$;*MHC-II*$^{(-/-)}$ mice (Fig. 9 *C*).

Discussion

By crossing the *TGF-β1*$^{(-/-)}$ genotype into an MHC class II–deficient background, we demonstrate that autoimmunity associated with TGF-β1-deficiency is dependent on the functional expression of class II molecules. In *MHC-II*$^{(-/-)}$ TGF-β1–deficient mice, there is a complete reversal of the inflammatory phenotype which is otherwise characteristic of *TGF-β1*$^{(-/-)}$ mice, with no evidence for the typically progressive cellular and humoral autoimmune process. More importantly, since the predominant phenotype of *TGF-β1*$^{(-/-)}$; *MHC-II*$^{(-/-)}$ mice is one of myeloid metaplasia, this approach reveals that the hematopoietic abnormalities present in *TGF-β1*$^{(-/-)}$ mice are not solely a response to systemic inflammation. The presence of similar manifestations in CD4$^+$ T cell–depleted *TGF-β1*$^{(-/-)}$;*MHC-II*$^{(+/+)}$ mice supports this conclusion. Our results clarify the mechanisms underlying the autoimmunity of *TGF-β1*$^{(-/-)}$ mice and strongly support the concept that endogenous TGF-β1 is a critical negative regulator of hematopoiesis, and particularly myelopoiesis.

Enhanced expression of MHC class II molecules is a prominent feature of several murine models of autoimmunity, including experimental allergic encephalomyelitis, where there is significantly increased detection of class II antigens within the central nervous system (36). More importantly, the ability of systemically administered TGF-β1 to ameliorate or inhibit

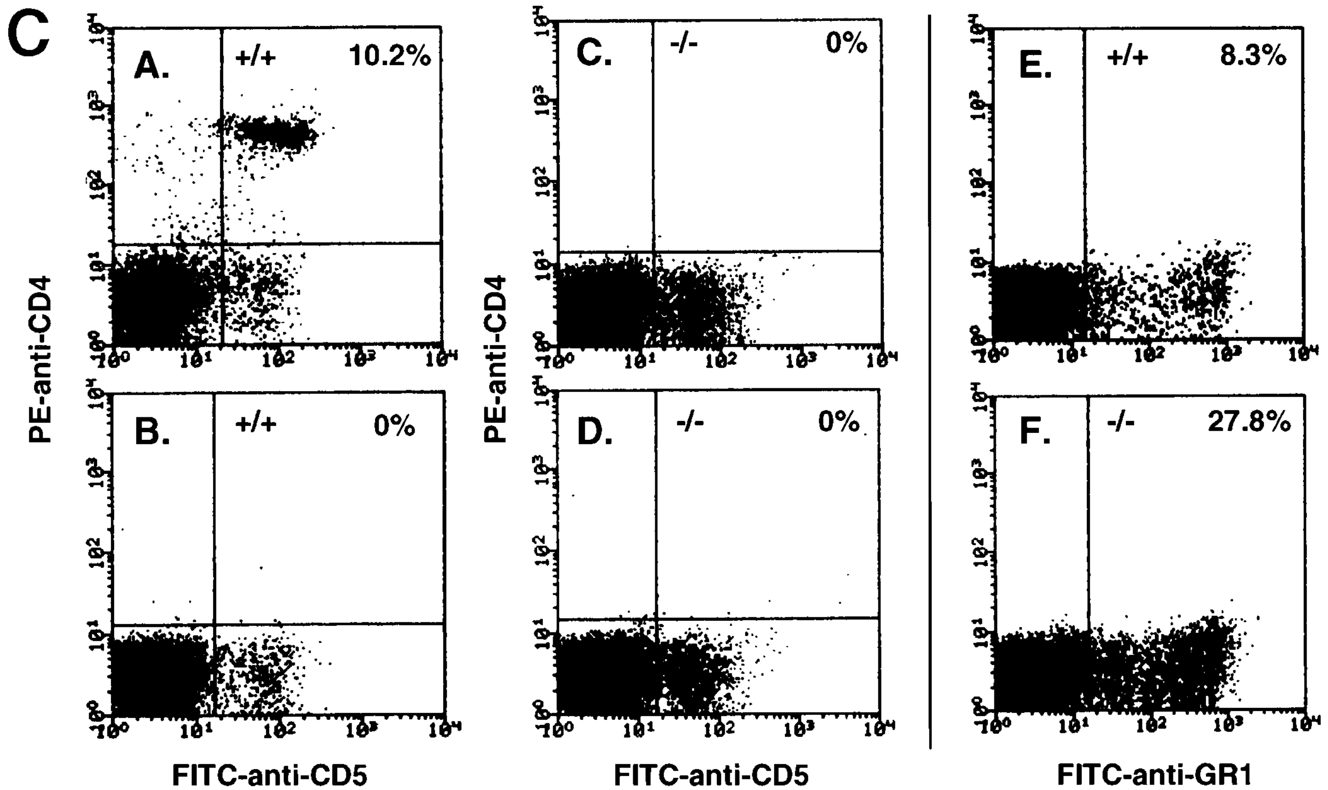

Figure 9.

the autoimmune disease coincides with a reduction in the local expression of MHC class II molecules (24). Indeed, TGF-β1 functions as a natural suppressor of the inflammatory process in this disease model, with substantially increased levels of TGF-β1 immunoreactivity present in brain at the onset of disease remission (37), and with increased disease severity after the administration of antibodies to the type 1 isoform of TGF-β (22).

Although TGF-β is known to have direct effects on transcription of class II MHC antigens (38), the enhanced expression observed in *TGF-β1$^{(-/-)}$* mice may be secondary to other factors released by lymphocytes, since the immunodetection of class II antigens in immune-deficient (SCID), *TGF-β1$^{(-/-)}$* mice appears to be normal, at least within the myocardium (19). Regardless of the mechanism driving increased class II antigen expression in *TGF-β1$^{(-/-)}$* mice, the inflammatory process does not evolve in the absence of these molecules, and all of the manifestations which characterize the process as autoimmune in nature are lost in *TGF-β1$^{(-/-)}$;MHC-II$^{(-/-)}$* mice, including the circulating autoantibodies and glomerular immune complex deposits. Finally, although we also reported that increased MHC class I expression is present in *TGF-β1$^{(-/-)}$* mice (16), it remains uncertain whether this may contribute to the autoimmunity of TGF-β1-deficiency, particularly since we continue to detect increased class I expression in our *TGF-β1$^{(-/-)}$;MHC-II$^{(-/-)}$* mice (data not shown).

It is important to note that *MHC-II$^{(-/-)}$* mice are also depleted of mature CD4^{+} T cells, rendering them deficient in cell-mediated immune responses (27). This feature of *MHC-*

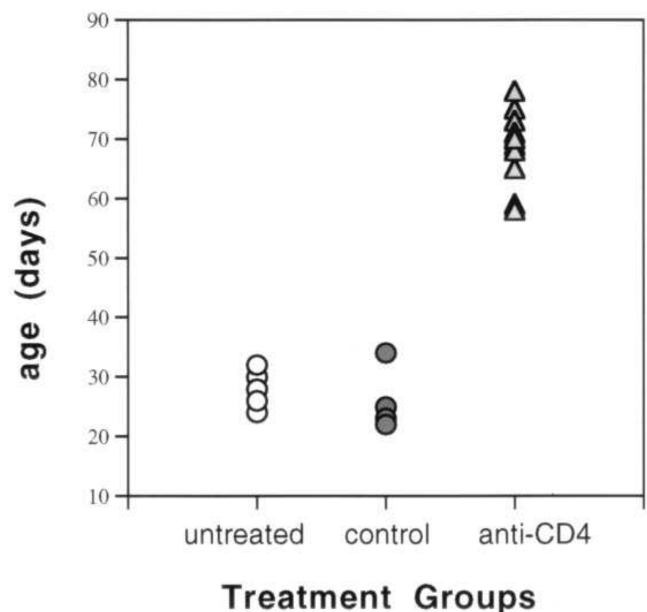

Figure 10. Increased survival of CD4-depleted *TGF-β1$^{(-/-)}$* mice. *TGF-β1$^{(-/-)}$* mice were followed with daily weights and killed when symptoms or weight loss developed. While mice receiving injections of control Ab49 (*filled circles*) exhibit a mean survival similar to untreated *TGF-β1$^{(-/-}$* mice (*open circles*), those treated with anti-CD4 antibodies (*filled triangles*) have a significant increase in their survival ($P < 0.0001$). Each symbol represents a single animal, with 5 each in untreated and control groups, and 10 in the anti-CD4 group.

$II^{(-/-)}$ mice may interfere with the expression of the TGF-β1–null phenotype independent of the absence of class II antigen expression on peripheral tissues (39). For example, helper T cells secrete a variety of cytokines involved in normal B cell differentiation and are thought to be necessary to initiate the transformation of B cells to mature, antibody-secreting plasma cells (40). Accordingly, MHC class II–deficient animals cannot generate antibodies of the IgG isotype in response to antigen challenge, as the switch from IgM to IgG isotypes requires the interaction between $CD4^{+}$ T cells and antigen-specific B cells. Such a defect may underlie the lack of circulating antinuclear antibodies in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice, as these are exclusively of the IgG isotype (15). The importance of $CD4^{+}$ T cells in the pathogenesis of the autoimmune phenotype is supported by immunohistochemical analyses of mononuclear cell infiltrates in *TGF-β1*$^{(-/-)}$ mice (these reveal a predominance of $CD4^{+}$ lymphocytes which are clearly absent in respective tissues of the *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice) (Nakabayashi, T., J. Letterio, N. Kong, N. Ogawa, H. Dang, and N. Talal, manuscript submitted for publication). Finally, our results show that in vivo depletion of $CD4^{+}$ T cells in *TGF-β1*$^{(-/-)}$ mice significantly impairs the development of autoimmunity, and directly demonstrates their role in the genesis of the *TGF-β1*$^{(-/-)}$ phenotype.

Perhaps the most significant aspect of this model is the apparent unmasking of the effects of TGF-β1 on hematopoiesis, which becomes prominent in the absence of inflammation in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice. Enhanced myelopoiesis is a component of the *TGF-β1*$^{(-/-)}$ phenotype in both *MHC-II*$^{(+/+)}$ and *MHC-II*$^{(-/-)}$ backgrounds, as evident from the presence of increased numbers of circulating granulocytes and monocytes, with a progressive extramedullary hematopoiesis typically accompanied by splenomegaly and lymphadenopathy. This model now provides substantial experimental evidence suggesting that this aspect of the phenotype is a direct consequence of the loss of specific negative regulatory effects of TGF-β1 on myelopoiesis and not the result of production of inflammatory cytokines from cells participating in the autoimmune process. These data support previous demonstrations of the inhibitory effects of TGF-β on the proliferation of hematopoietic stem cells and early progenitors, which presumably result from a reversible arrest in the G1 phase of the cell cycle (41). Long-term murine bone marrow cultures treated with antibodies to TGF-β contain more than three times the number of stem cells as control cultures (41), and the addition of even picomolar concentrations of TGF-β to these cultures has a profound effect on granulo-monocytic, erythroid, and megakaryocytic lineages (42). Similar effects have been demonstrated in vivo, with chronic systemic administration of TGF-β1 leading to a 50% reduction in red cell numbers and a $>$ 95% reduction in circulating platelets (43). One potential mechanism underlying these observations is the regulation of receptor expression for colony-stimulating factors (44, 45). This may be an important function of TGF-β1 in vivo and is suggested by the increased in vitro sensitivity of *TGF-β1*$^{(-/-)}$*; MHC-II*$^{(-/-)}$ marrow cultures to GM-CSF and IL-3 (data not shown).

It is possible that the absence of class II MHC antigens may also contribute to the dysregulation of hematopoiesis in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice. While MHC class II–deficient mice display no apparent abnormalities with respect to normal hematopoiesis, the expression of class II antigens on hematopoietic precursor cells and their contribution to normal myelopoiesis remains controversial. Expression of class II antigens can be found on committed hematopoietic progenitors, particularly those of the granulocyte-monocyte-erythrocyte and megakaryocyte lineages. Maturation along the granulocytic lineage leads to the loss of cell surface expression of MHC class II antigens, though functionally mature monocytes and macrophages maintain class II expression (46). Antibodies specific for MHC class II inhibit the growth of primitive hematopoietic progenitor cells in vitro (47), and when administered in vivo they impair the survival of autologous marrow grafts (46). These data suggest that the presence of class II molecules on stem cells may play an important part in any potentially MHC-restricted cell collaboration in hematopoiesis (48). The relevance of such interactions in the establishment of the *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ phenotype is supported by the apparent absence of characteristic myeloid hyperplasia in the SCID (T and B lymphocyte–depleted) *TGF-β1*$^{(-/-)}$ mice (19). Of interest, our CD4-depleted *TGF-β1*$^{(-/-)}$ mice continue to demonstrate increased myelopoiesis as indicated by percentages of $GR1^{+}$ cells in various hematopoietic organs (Fig. 9 *C*), although the process appears to progress less rapidly than in *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice. Thus, alterations in normal patterns of MHC class II antigen expression, coupled with the loss of negative regulatory effects of TGF-β1, may act in concert to produce the more pronounced shift towards enhanced myelopoiesis observed in the *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice.

In conclusion, our results demonstrate a dependence on class II antigens for the expression of the autoimmune phenotype associated with TGF-β1-deficiency. While these data suggest that the altered expression of these molecules observed in *TGF-β1*$^{(-/-)}$ mice contributes to the genesis of the inflammatory process, they also firmly establish an essential role for the type 1 isoform of TGF-β in control of leukocyte maturation and function. The presence of progressive myeloid hyperplasia, which is the predominant phenotype in the absence of autoimmunity, confirms expectations that loss of the negative regulatory effects of TGF-β1 would produce significant abnormalities in hematopoiesis. Moreover, the distinct similarity between this phenotype and pathologies such as agnogenic myeloid metaplasia in humans suggests that alterations in TGF-β1 expression may be involved in this and other myeloproliferative disorders. The *TGF-β1*$^{(-/-)}$*;MHC-II*$^{(-/-)}$ mice should now serve as a model system in which to investigate further the isoform-specific activities of TGF-β1 in such processes. Finally, with such profound effects on the maintenance of immunological homeostasis and immune tolerance, TGF-β1 clearly must be considered a candidate for use in the treatment of autoimmune diseases and other human conditions associated with a dysregulated immune response.

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References

1. Bombara, C., and R. Ignotz. 1992. TGF-beta inhibits proliferation of and promotes differentiation of human promonocytic leukemia cells. *J. Cell. Physiol.* 153:30–37.

2. Gamble, J.R., Y. Khew-Goodall, and M.A. Vadas. 1993. Transforming growth factor-β inhibits E-selectin expression on human endothelial cells. *J. Immunol.* 150:4494–4503.

3. Wahl, S.M., J.B. Allen, B.S. Weeks, H.L. Wong, and P.E. Klotman. 1993. Transforming growth factor β enhances integrin expression and type IV collagenase secretion in human monocytes. *Proc. Natl. Acad. Sci. USA.* 90:4577–4581.

4. Tsunawaki, S., M. Sporn, A. Ding, and C. Nathan. 1988. Deactivation of macrophages by transforming growth factor-β. *Nature (Lond.).* 334:260–262.

5. Vodovotz, Y., C. Bogdan, J. Paik, Q.-W. Xie, and C. Nathan. 1993. Mechanisms of suppression of macrophage nitric oxide release by transforming growth factor β. *J. Exp. Med.* 178:605–613.

6. McCartney-Francis, N.L., and S.M. Wahl. 1994. Transforming growth factor β: a matter of life and death. *J. Leukocyte Biol.* 55:401–409.

7. Wahl, S.M. 1992. TGF-β in inflammation. A cause and a cure. *J. Clin. Immunol.* 12:61–74.

8. Wahl, S.M. 1994. Transforming growth factor β: the good, the bad, and the ugly. *J. Exp. Med.* 180:1587–1590.

9. Fontana, A., D.B. Constam, K. Frei, U. Malipiero, and H.W. Pfister. 1992. Modulation of the immune response by transforming growth factor beta. *Int. Arch. Allergy Immunol.* 99:1–7.

10. Letterio, J.J., and A.B. Roberts. 1996. Transforming growth factor-β1-deficient mice: identification of isoform-specific activities *in vivo. J. Leukocyte Biol.* 59:769–774.

11. Kulkarni, A.B., J.M. Ward, A.G. Geiser, J.J. Letterio, K.L. Hines, M. Christ, R.N. D'Souza, C. Huh, A.B. Roberts, M.B. Sporn, et al. 1994. TGF-β1 knockout mice: immune dysregulation and pathology. *In* Molecular Biology of Haematopoiesis. N.G. Abraham, R.K. Shadduck, A.S. Levine, and F. Takaku, editors. Intercept, Paris. 749–757.

12. Schull, M.M., A.B. Kier, R.J. Diebold, Y. Moying, and T. Doetschman. 1994. The importance of transforming growth factor β1 in immunological homeostasis, as revealed by gene ablation in mice. *In* Overexpression and Knockout of Cytokines in Transgenic Mice. Academic Press Ltd., London. 135–159.

13. Schull, M.M., I. Ormsby, A.B. Kier, S. Pawlowski, R.J. Diebold, M. Yin, R. Allen, C. Sidman, B. Proetzel, D. Calvin, et al. 1992. Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature (Lond.).* 359:693–699.

14. 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.* 90:770–774.

15. Dang, H., A.G. Geiser, J.J. Letterio, T. Nakabayashi, L. Kong, G. Fernandes, and N. Talal. 1995. SLE-like autoantibodies and Sjögren's syndrome-like lymphoproliferation in TGF-β1 knockout mice. *J. Immunol.* 155: 3205–3212.

16. Geiser, A.G., J.J. Letterio, A.B. Kulkarni, S. Karlsson, A.B. Roberts, and M.B. Sporn. 1993. Transforming growth factor β1 (TGF-β1) controls expression of major histocompatibility genes in the postnatal mouse: aberrant histocompatibility antigen expression in the pathogenesis of the TGF-β1 null mouse phenotype. *Proc. Natl. Acad. Sci. USA.* 90:9944–9948.

17. Christ, M., N.L. McCartney-Francis, A.B. Kulkarni, J.M. Ward, D.E. Mizel, C.L. Mackall, R.E. Gress, K.L. Hines, H. Tian, S. Karlsson, and S.M. Wahl. 1994. Immune dysregulation in TGF-β1-deficient mice. *J. Immunol.* 153: 1936–1946.

18. Hines, K.L., A.B. Kulkarni, J.B. McCarthy, H. Tian, J.M. Ward, M. Christ, N.L. McCartney-Francis, L.T. Furcht, S. Karlsson, and S. Wahl. 1994. Synthetic fibronectin peptides interrupt inflammatory cell infiltration in transforming growth factor β1 knockout mice. *Proc. Natl. Acad. Sci. USA.* 91:5187–5191.

19. Diebold, R.J., M.J. Eis, M. Yin, I. Ormsby, G.P. Boivin, B.J. Darrow, J.E. Saffitz, and T. Doetschman. 1995. Early-onset multifocal inflammation in the transforming growth factor β1-null mouse is lymphocyte mediated. *Proc. Natl. Acad. Sci. USA.* 92:12215–12219.

20. Yaswen, L., A.B. Kulkarni, T. Frederickson, B. Mittleman, R. Schiffmann, S. Payne, G. Longenecker, E. Mozes, and S. Karlsson. 1995. Autoimmune manifestations in the TGF-β1 knockout mouse. *Blood.* 87:1439–1445.

21. Miller, A., O. Lider, A.B. Roberts, M.B. Sporn, and H.L. Weiner. 1992. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both *in vitro* and *in vivo* immune responses by the release of transforming growth factor β after antigen-specific triggering. *Proc. Natl. Acad. Sci. USA.* 89: 421–425.

22. Racke, M.K., B. Cannella, P. Albert, M. Sporn, C.S. Raine, and D.E. McFarlin. 1992. Evidence of endogenous regulatory function of transforming growth factor-β1 in experimental allergic encephalomyelitis. *Int. Immunol.* 4: 615–620.

23. Lowrance, J.H., F.X. O'Sullivan, T.E. 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.

24. Racke, M.K., S. Dhib-Jalbut, B. Cannella, P.S. Albert, C.S. Raine, and D.E. McFarlin. 1991. Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor-β1. *J. Immunol.* 146:3012–3017.

25. Brandes, M.E., J.B. Allen, Y. Ogawa, and S.M. Wahl. 1991. Transforming growth factor beta 1 suppresses acute and chronic arthritis in experimental animals. *J. Clin. Invest.* 87:1108–1113.

26. Sinha, A.A., T. Lopez, and H.O. McDevitt. 1990. Autoimmune diseases: the failure of self tolerance. *Science (Wash. DC).* 248:1380–1388.

27. Grusby, M.J., R.S. Johnson, V.E. Papaioannou, and L.H. Glimcher. 1991. Depletion of CD4+ T cells in major histocompatibility complex II-deficient mice. *Science (Wash. DC).* 253:1417–1420.

28. Dialynas, D.P., Z.S. Quan, K.A. Wall, A. Pierres, J. Quintans, M.R. Loken, M. Pierres, and F.W. Fitch. 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J. Immunol.* 131:2445–2450.

29. Pierres, A., P. Naquet, A. van Agthoven, F. Bekkhoucha, F. Denizot, Z. Mishal, A.M. Verhulst, and M. Pierres. 1984. A rat anti-mouse T4 monoclonal antibody (H129.19) inhibits the proliferation of Ia-reactive T cell clones and delineates two phenotypically distinct ($T4^+$, Lyt-$2,3^-$, and $T4^-$, Lyt-$2,3^+$) subsets among anti-Ia cytolytic T cell clones. *J. Immunol.* 132:2775–2782.

30. Tan, E.M. 1982. Autoantibodies to nuclear antigens: their immunobiology and medicine. *Adv. Immunol.* 33:167–172.

31. Eisenberg, R.A., S.Y. Craven, R.W. Warren, and P.L. Cohen. 1987. Stochastic control of anti-Sm autoantibodies in MRL/Mp-*lpr/lpr* mice. *J. Clin. Invest.* 80:691–697.

32. Boivin, G.P., B.A. O'Toole, I.E. Orsmby, R.J. Diebold, M.J. Eis, T. Doetschman, and A.B. Kier. 1995. Onset and progression of pathological lesions in transforming growth factor-β1-deficient mice. *Am. J. Pathol.* 146:276–288.

33. Wofsy, D., J.A. Ledbetter, P.L. Hendler, and W.E. Seaman. 1985. Treatment of murine lupus with monoclonal anti-T cell antibody. *J. Immunol.* 134: 852–859.

34. Wofsy, D., and W.E. Seaman. 1985. Successful treatment of autoimmunity in NZB/NZW F1 mice with monoclonal antibody to L3T4. *J. Exp. Med.* 161:378–391.

35. Ranges, G.E., S. Sriram, and S.M. Cooper. 1985. Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J. Exp. Med.* 162:1105–1112.

36. Sobel, R.A., B.W. Blanchette, A.K. Bhan, and R.B. Colvin. 1984. The immunopathology of acute experimental allergic encephalomyelitis. II. Endothelial cell Ia expression increases prior to inflammatory cell infiltration. *J. Immunol.* 132:2402–2407.

37. Khoury, S.J., 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 beta, interleukin 4, and prostaglandin E expression in the brain. *J. Exp. Med.* 176:1355–1364.

38. Reimold, A.M., C.J. Kara, J.W. Rooney, and L.H. Glimcher. 1993. Transforming growth factor β1 repression of the HLA-DRa gene is mediated by conserved proximal promoter elements. *J. Immunol.* 151:4173–4182.

39. Jameson, B.A., J.M. McDonnell, J.C. Marini, and R. Korngold. 1994. A rationally designed CD4 analogue inhibits experimental allergic encephalomyelitis. *Nature (Lond.).* 368:744–746.

40. Hilbert, D.M., M.-Y. Shen, U.R. Rapp, and S. Rudikoff. 1995. T cells induce terminal differentiation of transformed B cells to mature plasma cell tumors. *Proc. Natl. Acad. Sci. USA.* 92:649–653.

41. Waegell, W.O., H.R. Higley, P.W. Kincade, and J.R. Dasch. 1994. Growth acceleration and stem cell expansion in Dexter-type cultures by neutralization of TGF-β. *Exp. Hematol.* 22:1051–1057.

42. Jacobsen, S.E., J.R. Keller, F.W. Ruscetti, P. Kondaiah, A.B. Roberts, and L.A. Falk. 1991. Bidirectional effects of transforming growth factor-β (TGF-β) on colony-stimulating factor-induced human myelopoiesis in vitro: differential effects of distinct TGF-β isoforms. *Blood.* 78:2239–2245.

43. Carlino, J.A., H.R. Higley, J.R. Creson, P.D. Avis, and Y. Ogawa. 1992. Transforming growth factor-β1 systematically modulates granuloid, erythroid, lymphoid, and thrombocytic cells in mice. *Exp. Hematol.* 20:943–949.

44. Heinrich, M.C., D.C. Dooley, and W.W. Keeble. 1995. Transforming growth factor β1 inhibits expression of the gene products for steel factor and its receptor (c-kit). *Blood.* 85:1769–1780.

45. Jacobsen, S.E., F.W. Ruscetti, A.B. Roberts, and J.R. Keller. 1993. TGF-β is a bidirectional modulator of cytokine receptor expression on murine bone marrow cells. Differential effects of TGF-β1 and TGF-β3. *J. Immunol.* 151:4534–4539.

46. Deeg, H.J., C. Beckham, R. Huss, D. Myerson, H. Greinix, F.R. Appelbaum, T. Graham, F. Schuening, and R. Storb. 1994. Rescue from anti-MHC class II antibody-mediated marrow graft failure by c-kit ligand. *Blood.* 83:2352–2359.

47. Greinix, H.T., R. Storb, and S.H. Bartelmez. 1992. Specific growth inhibition of primitive hematopoietic progenitor cells mediated through monoclonal antibody binding to major histocompatibility class II molecules. *Blood.* 80:1950–1956.

48. Torok-Storb, B., and J.A. Hansen. 1982. Modulation of in vitro BFU-E growth by normal Ia-positive T cells is restricted by HLA-DR. *Nature (Lond.).* 298:473–478.