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Genetics

Transforming growth factor β_1 null mutation in mice causes excessive inflammatory response and early death

(homologous recombination/embryonic stem cells/inflammation/autoimmunity)

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ABSTRACT **To delineate specific developmental roles of transforming growth factor β_1 (TGF-β_1) we have disrupted its cognate gene in mouse embryonic stem cells by homologous recombination to generate TGF-β_1 null mice. These mice do not produce detectable amounts of either TGF-β_1 RNA or protein. After normal growth for the first 2 weeks they develop a rapid wasting syndrome and die by 3–4 weeks of age. Pathological examination revealed an excessive inflammatory response with massive infiltration of lymphocytes and macrophages in many organs, but primarily in heart and lungs. Many lesions resembled those found in autoimmune disorders, graft-vs.-host disease, or certain viral diseases. This phenotype suggests a prominent role for TGF-β_1 in homeostatic regulation of immune cell proliferation and extravasation into tissues.**

Since the initial characterization of transforming growth factor β (TGF-β) in 1983 as a homodimeric, 25-kDa peptide, there has been a rapid advance in knowledge of its biological roles (for reviews, see refs. 1 and 2). TGF-βs are known to be intimately involved in many cellular processes such as cell proliferation and differentiation, embryonic development, extracellular matrix formation, bone development, wound healing, hematopoiesis, and immune and inflammatory cell response (1, 2). The modulation of immune and inflammatory responses by TGF-βs includes (*i*) inhibition of proliferation of all T-cell subsets, (*ii*) inhibitory effects on proliferation and function of B lymphocytes, (*iii*) down-regulation of natural killer cell activity and the T-cell response, (*iv*) regulation of cytokine production by immune cells, and (*v*) regulation of macrophage function (3–9).

Five distinct TGF-β genes have been identified in vertebrates and three of these (TGF-β_1, TGF-β_2, and TGF-β_3) are expressed in mammals. Each of the three isoforms has been highly conserved throughout evolution, suggesting specific roles for each (1, 2). These three isoforms share a high degree of amino acid sequence homology in the mature domain, are often coexpressed and colocalized, and have qualitatively similar actions on tissue culture cells (1, 2). Therefore, it has been difficult to define the precise biological role of individual TGF-β isoforms. To delineate and define the specific *in vivo* role of TGF-β_1, we disrupted the murine TGF-β_1 gene in embryonic stem (ES) cells by homologous recombination (for reviews, see refs. 10 and 11). The targeted cells were subsequently used to generate mice with a loss-of-function mutation at the TGF-β_1 locus. Although the TGF-β_1 null mutation in the homozygous state causes some intrauterine lethality, more than one-third of the fetuses develop to term and appear clinically normal at birth. After 2 weeks these mice develop a wasting syndrome and die ≈1–2 weeks later. Massive inflammatory lesions are seen in many organs, including the lungs (vasculitis, perivascular cuffing, and interstitial pneumonia) and heart (endocarditis and myocarditis), suggesting an uncontrolled inflammatory response that leads to premature death.

MATERIALS AND METHODS

Constructs. A 5.7-kb *Bgl* II genomic fragment containing the first two exons of TGF-β_1 from the previously described clone pB2 (12) was subcloned into the modified Bluescript KS vector (Stratagene) in which the *Asp* 718 site was converted to a *Sfi* I site to generate pB2-3. A 560-bp sequence spanning part of the first exon (154 bp of coding sequence) and intron was deleted following *Asp* 718 digestion of pB2-3, and the phosphoglycerate kinase (PGK)-neomycin resistance (neo) gene (13) was inserted. The targeting vector, pTC-1, also contained a PGK-driven herpes simplex virus thymidine kinase gene (PGK-HSVtk) at the 3′ end (see Fig. 1). Both marker genes also contain PGK poly(A) signals (13).

ES Cell Culture, Transfection, and Selection. The CCE ES cell line (a generous gift from E. Robertson, Columbia University) was cultured on mitomycin-treated STO feeder layers in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (HyClone) and 10% newborn calf serum (GIBCO) as described (14). ES cells were grown to 70% confluency, trypsinized, and resuspended in phosphate-buffered saline (PBS; Ca^{2+} and Mg^{2+} free) at 10^7 cells per ml. Twenty micrograms of targeting vector, pTC-1, linearized at the 5′ end with *Not* I, was mixed with 0.8 ml of the cell suspension and electroporated at 500 μF and 240 V (Bio-Rad Gene Pulser). The cells were plated on two 10-cm Petri dishes containing STO feeders. Selection was applied after 24 hr with 400 μg of G418 (Geneticin) per ml or G418 and 2 μM gancyclovir (GANC). The cells were grown for another 8–10 days with daily medium changes, and robust colonies were counted, cloned, and expanded for further analysis.

DNA and RNA Analysis. Individual colonies and pools of G418- and GANC-resistant CCE ES cells were screened by "nested PCR" (15, 16) to identify the homologous recombinant clones. The temperature cycles consisted of denaturation at 94°C for 1 min, annealing at 60°C for 1.5 min, and

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Abbreviations: ES, embryonic stem; G418, Geneticin; GANC, gancyclovir; GVHR, graft-vs.-host reaction; HSV-tk herpes simplex virus thymidine kinase; neo, neomycin; PGK, phosphoglycerate kinase; TGF-β, transforming growth factor β; H&E, hematoxylin/eosin.

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amplification at 72°C for 1 min. One set of 40 such cycles was used. The positions of various primers used in this assay are shown in Fig. 1. The first set of amplification was performed using the primers P3 (CAGAGTCTGAGACCAGCCGCCG) and P4 (ACCTGCGTGCAATCCATCTTGTTCAATGG). The second set of amplification was performed using the nested primers P1 (TCACCGTCGTGGACACTCGAT) and P2 (TCCATCTGCACGAGACTAGT) and 5 μl of a 100-fold dilution of the amplification product from the first set. Southern and Northern blot analyses were performed using standard techniques (12, 17).

Generation of Chimeras and Histopathological Analysis. All manipulations were performed using C57BL/6J recipients as described (18). Mice were housed in a double-barrier facility. By serological testing, the TGF-β_1 (+/+), TGF-β_1 (+/−), and TGF-β_1 (−/−) mice were free of antibodies to the common murine viruses and pathogens (adenovirus, cab bacillus, ectomellia, rotavirus, GD VII, lymphocytic choriomeningitis, hepatitis virus, mycoplasma, pneumonia virus of mice, REO 3, Sendai virus, minute virus of mice, polyoma, mouse cytomegalovirus, parasites, and bacteria). Tissues from 14 TGF-β_1 (−/−) mice and 22 TGF-β_1 (+/+) or (+/−) littermates were fixed in 10% neutral buffered formalin and embedded in paraffin. Selected tissues were placed in Bouin's fixative. Five-micron sections were cut and stained with hematoxylin/eosin (H&E) and analyzed for histopathology. TGF-β_1 was localized in formalin-fixed tissue sections following a protocol similar to that previously described (19, 20). The rabbit TGF-β_1 antibody was generated against a peptide corresponding to amino acids 267–278 (19). Avidin-biotin immunohistochemistry was performed on Bouin's-fixed tissues for Mac-2 antigen (Mac-2 antibody was obtained from the American Type Culture Collection TIB 166 hybridoma culture; refs. 21 and 22) and mouse immunoglobulins using an anti-mouse IgG antibody (Vector Laboratories Vectastain mouse elite kit). The antibodies were used at optimal dilutions, which did not produce nonspecific background staining.

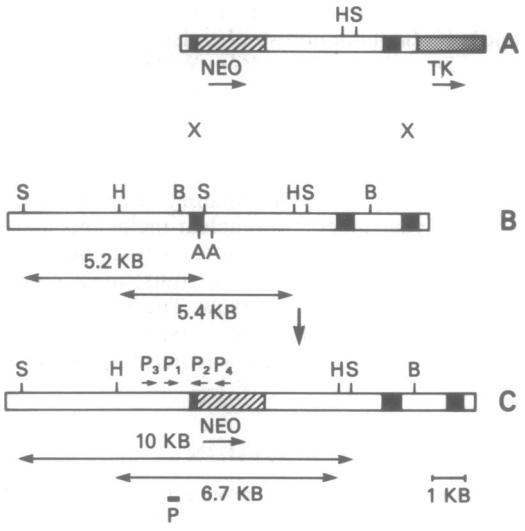

FIG. 1. Strategy for targeted disruption of the TGF-β_1 gene: Schematic diagram showing (*A*) the structure of targeting vector, pTC-1 (A), parental TGF-β_1 gene (*B*), and the predicted structure of the targeted TGF-β_1 gene (*C*). The TGF-β_1 exons are shown as solid boxes, introns and the promoterless region as open boxes, and the neo and tk sequences as hatched boxes. *Sac* I (S), *Hin*dIII (H), *Bgl* II (B), and *Asp* 718 (A) sites and distances between these sites are indicated in the restriction map of the TGF-β_1 gene. A 0.56-kb sequence between *Asp* 718 sites was deleted and the neo marker gene was inserted at the deletion. A 3-kb plasmid sequence at the 3′ end of the vector is not shown. The sizes of the *Sac* I and *Hin*dIII fragments in the normal and the targeted alleles are marked by double-headed arrows. The 5′ flanking probe used for the Southern analysis is represented by a thick line (P). The positions of the primers used for PCR analysis are shown by the short arrows, P1–P4.

RESULTS

Targeting of the TGF-β_1 Gene. The 5′ end of the TGF-β_1 gene was selected to construct the homologous recombination vector pTC-1 (Fig. 1), since there is no significant homology between the three mammalian TGF-β isoforms in this region (1, 2) and the entire gene (including the 5′ part that codes for the TGF-β_1 precursor) is disrupted by this approach. The vector is of the replacement type containing 5.2 kb of homology, 500 bp upstream and 4.7 kb downstream of the neo gene inserted in exon 1. Insertion of the neomycin resistance (neo) gene in this position disrupts the open reading frame of the TGF-β_1 gene. The 560-bp deletion in the TGF-β_1 sequences results in the loss of the *Sac* I site in the first intron. In addition to the neo marker gene, the pTC-1 vector also contains the HSV-tk gene at the 3′ end of the TGF-β_1 sequence for selection against random integration events. The double selection with G418 and GANC enriches the selection of cells that have undergone homologous recombination (23).

After electroporation of 8×10^7 cells and drug selection, 1700 G418-resistant clones were generated. Of these, 240 were resistant to G418 and GANC and they were screened by nested PCR. DNAs from 5–7 clones were pooled and analyzed. Individual clones from the PCR-positive pools were then further analyzed by PCR and confirmed by Southern blotting. Four individual clones were PCR positive and Southern blot analysis of genomic DNA confirmed gene targeting of these clones. Hybridization with the 5′ flanking probe displayed the novel 6.7-kb *Hin*dIII fragment for the disrupted allele and the 5.4-kb fragment for the wild-type allele (data not shown). Additionally, *Sac* I digestion of DNA from the targeted clones demonstrated a novel 10-kb fragment for the disrupted allele and a 5.2-kb fragment for the wild-type allele (data not shown). A single integration event was confirmed in all four clones by hybridization to the neo probe and absence of any rearrangement of the targeting vector by hybridization with the TGF-β_1 internal probe (data not shown).

Intrauterine or Premature Death of TGF-β_1 (−/−) Mice. Two of four clones generated germ-line chimeras whose offspring were heterozygous for the disrupted TGF-β_1 locus. The heterozygous mice were found to be normal and gained weight at the same rate as the normal mice. The heterozygous mice were then interbred to generate TGF-β_1 (−/−) mice. The distribution of mice in the first 139 pups delivered from 14 females was 48 (35%) TGF-β_1 (+/+), 77 (55%) TGF-β_1 (+/−), and 14 (10%) TGF-β_1 (−/−), suggesting considerable intrauterine lethality of the homozygous null genotype. Homozygosity of the disrupted TGF-β_1 locus in the TGF-β_1 (−/−) mice was confirmed by Southern analysis of the tail DNA. The tail DNA from the TGF-β_1 (−/−) mice showed only the novel size fragments for the disrupted allele but not the normal-size fragments for the wild-type allele using *Hin*dIII and *Sac* I digestion (Fig. 2). The TGF-β_1 (−/−) pups had the same birth weight as that of normal or heterozygous pups and their rate of weight gain for the first 10–14 days was similar to that of other littermates. However, they started losing weight thereafter, and, by the end of 21 days, their body weight was almost half that of their littermate controls. The wasted TGF-β_1 (−/−) mice had a disheveled appearance and died by 3–4 weeks of age. The illness started before weaning. Gross gastrointestinal manifestations (including diarrhea) were not seen and milk was found in the alimentary canal at death. None of the normal or heterozygous mice exhibited these symptoms.

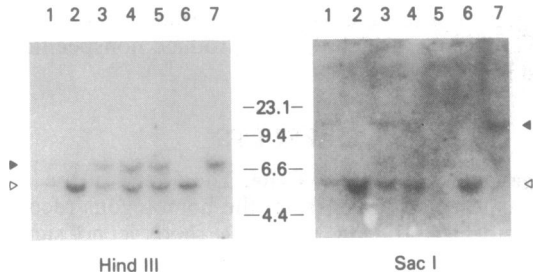

FIG. 2. Southern blot analysis of tail DNA from seven F_2 littermates. Tail DNA preparations were digested with either *Hin*dIII (*A*) or *Sac* I (*B*) and analyzed by Southern blot analysis with a ^{32}P-labeled 5′ flanking probe. The 6.7-kb *Hin*dIII fragment and the 10.0-kb *Sac* I fragment representing the targeted alleles are marked with the solid arrowheads. These fragments consistently hybridized to the neo probe. Lane 1 in the *Hin*dIII digest and lane 5 in the *Sac* I digest show a weak signal.

The Disrupted TGF-β_1 Locus Represents a Null Allele. The disruption of the TGF-β_1 locus and lack of TGF-β_1 expression were further confirmed by Northern analysis of the mRNA levels in several tissues (Fig. 3). As a control, TGF-β_3 mRNA expression was examined and it was found to be similar in the TGF-β_1 (−/−) mouse (except in the spleen) and the littermate controls. The steady-state mRNA levels of TGF-β_2 were not substantially altered either (data not shown). All of the tissues analyzed failed to show any detectable TGF-β_1 message even though expression in TGF-β_1 (+/+) and TGF-β_1 (+/−) mice was high. Several tissues from three mice (same litter) representing all genotypes [TGF-β_1 (+/+), TGF-β_1 (+/−), and TGF-β_1 (−/−)] were analyzed by immunohistochemistry using specific antibody to TGF-β_1. The TGF-β_1 protein was not present in any of the tissues examined from the TGF-β_1 (−/−) mouse (e.g., see Fig. 4 *H* and *I* for pancreas; data for other tissues not shown) when compared with negative controls [TGF-β_1 (+/−) samples stained with normal rabbit serum IgG]. TGF-β_1 levels were also measured in acid/ethanol extract of whole blood (normally blood platelets are a rich source of TGF-β_1) from another TGF-β_1 (−/−) mouse (different litter)

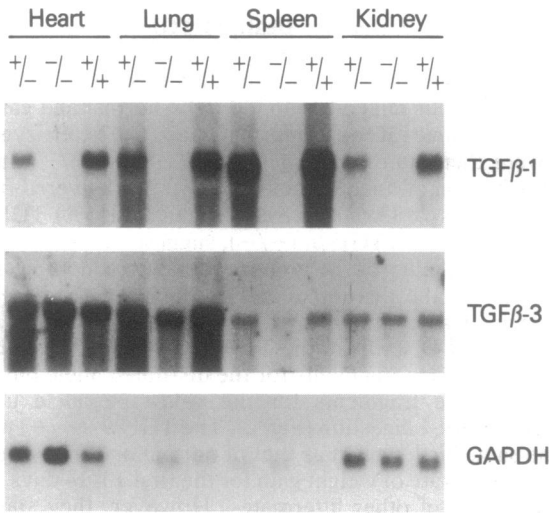

FIG. 3. TGF-β_1 mRNA levels in heart, lung, spleen, and kidney of the TGF-β_1 (+/−), TGF-β_1 (−/−), and TGF-β_1 (+/+) littermates. Fifteen micrograms of total RNA was loaded on the gel and subjected to Northern blot analysis with ^{32}P-labeled TGF-β_1 and TGF-β_3 cDNAs and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. GAPDH levels were similar for all genotypes in each tissue. A longer exposure was used to estimate this in lungs and spleen.

and compared with levels in a littermate control [TGF-β_1 (+/+)] using a quantitative sandwich ELISA (24). The TGF-β_1 (−/−) mice had undetectable TGF-β_1 levels (at least 100-fold lower than the control and detection limit of the assay was 0.5 pmolar; David Danielpour, personal communication).

Pathological Changes Indicate an Excessive Inflammatory Response. Pathological examination of 14 TGF-β_1 (−/−) mice between ages 10 and 21 days revealed massive inflammatory lesions in many organs. All animals had lesions in the heart and lungs (Fig. 4 *A–G*) but involvement of other organs was variable from one mouse to another. Lesions were also found in spleen, mediastinal lymph nodes, pancreas, colon, and salivary glands (sialoadenitis) of the TGF-β_1 (−/−) mice but not the TGF-β_1 (+/−) or (+/+) littermates. In the heart (Fig. 4 *D–G*), these lesions included atrial endocarditis and myocarditis in all TGF-β_1 (−/−) mice studied, with pericarditis and atrial thrombosis in some cases. In the lungs, lesions included vasculitis (phlebitis) and interstitial pneumonia (Fig. 4 *A–C*). In the mediastinal lymph nodes and spleen there was proliferation of immunoblasts and lymphoblasts in B- and T-cell zones (data not shown). Vascular and focal inflammatory lesions were also seen in the pancreas of two TGF-β_1 (−/−) mice and the salivary glands of several TGF-β_1 (−/−) mice (data not shown). Some mice had mild colonic and gastric necrosis. However, renal glomeruli and other areas of the kidney were normal. The inflammatory infiltrates seen in most tissues were generally composed of large macrophages (Fig. 4 *A*, *B*, *E*, and *F*), small lymphocytes, immunoblasts (Fig. 4 *B* and *C*), and some plasma cells. Histopathological analysis of the inflammatory lesions in the TGF-β_1 (−/−) mice did not reveal significant numbers of neutrophils. In the heart of one mouse, syncytial cells of the mononuclear phagocyte were seen (Fig. 4*F*). Rare syncytial bronchial epithelial cells were seen in the lungs of two mice. In Bouin's-fixed tissues, cardiac and pulmonary lesions contained predominantly Mac-2-immunoreactive macrophages using avidin-biotin immunohistochemistry and mouse monoclonal Mac-2 antibody (Fig. 4*G*). With biotinylated anti-mouse IgG, we demonstrated immunoglobulin-containing cells in lung (Fig. 4*C*), heart lesions, spleen, and mediastinal nodes (data not shown). Normal mice of this age have few of these inflammatory cells in these tissues. Nucleoli and nucleoli-like bodies of the inflammatory macrophages were often large and resembled intranuclear inclusions caused by some DNA viruses (25). No significant histological lesions were seen in the small intestine, thymus, most connective tissues, brain, skin, and bone marrow. The bone marrow cells were cytologically normal without evidence of malignant transformation, and hyperplasia was not detected histologically.

DISCUSSION

We have generated transgenic mouse lines with a null mutation at the TGF-β_1 locus by gene targeting in ES cells. Neither TGF-β_1 mRNA nor protein can be detected in the TGF-β_1 (−/−) mice, indicating that we have successfully generated a TGF-β_1 null mutant. Less than half of the expected number of TGF-β_1 (−/−) mice are born, suggesting considerable intrauterine lethality. The TGF-β_1 (−/−) mice have widespread inflammatory reaction in multiple organs causing cardiopulmonary death at ≈3–4 weeks of age due to the profound pathology in heart and lungs. This phenotype suggests a prominent role for TGF-β_1 in homeostatic regulation of immune cell proliferation and extravasation into tissues.

The histopathology associated with the TGF-β_1 (−/−) phenotype suggests that the absence of TGF-β_1 in these mice may facilitate a generalized activation of the immune system

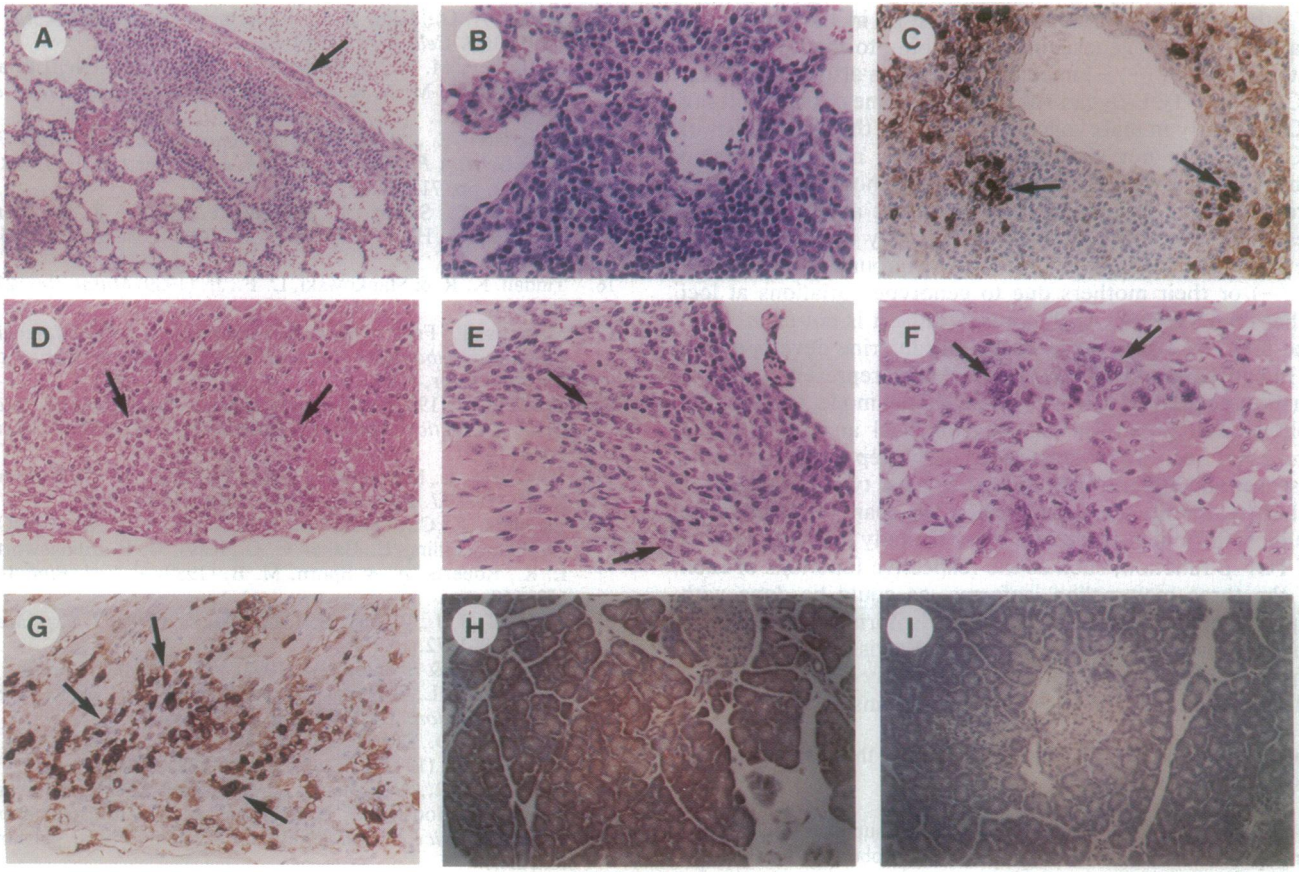

FIG. 4. Histopathological analysis on lung and heart tissues of TGF-β_1 (−/−) mice and littermate controls and immunohistochemical detection of TGF-β_1 in pancreas. (*A*) Lung of a TGF-β_1 (−/−) mouse showing severe phlebitis with perivascular cuffing and interstitial pneumonia. Note arterial wall (arrow) is normal. (H&E; ×70.) (*B*) Lung from a TGF-β_1 (−/−) mouse showing phlebitis. Inflammatory cells are lymphocytes and macrophages. (H&E; ×175.) (*C*) Immunohistochemistry for mouse IgG with Bouin's-fixed lung from the TGF-β_1 (−/−) mouse. Perivascular inflammatory cells include plasma cells (arrows) and lymphocytes. (Hematoxylin; ×175.) (*D*) Heart of a TGF-β_1 (−/−) mouse showing endocarditis and myocarditis. Inflammatory cells are predominantly macrophages (arrows). (H&E; ×175.) (*E*) Heart of a TGF-β_1 (−/−) mouse showing myocarditis and endocarditis. Note lymphocytes and macrophages (arrows) in the lesion. (H&E; ×175.) (*F*) Myocarditis of the TGF-β_1 (−/−) mouse. Mononuclear inflammatory cells include some syncytial cells (arrows). (H&E; ×280.) (*G*) Immunohistochemistry of endocardial and myocardial lesions in a TGF-β_1 (−/−) mouse showing Mac-2 expression in cytoplasm and nucleus of mononuclear cells (arrows). (Hematoxylin; ×175.) For immunohistochemistry, sections were stained with an anti-TGF-β_1 antibody as described in the text. (*H* and *I*) Acinar cells in the pancreas are positive for TGF-β_1 in the TGF-β_1 (+/−) mouse (*H*), whereas no staining is detected in these organs of the TGF-β_1 (−/−) mouse (*I*). No staining was observed in these sections using normal rabbit serum IgG at 3 μg/ml. (Stained with peroxidase and counterstained with Mayer's hematoxylin; ×180.)

by stimuli that are unable to provoke disease in normal littermates. This is consistent with the reported role of TGF-β_1 as a potent immunosuppressor (9). Since TGF-β is known to antagonize the activity of interleukin (IL) 1, tumor necrosis factor α, interferon γ, and IL-6, the TGF-β_1 (−/−) mice are expected to have increased activity of these proinflammatory cytokines. Moreover, lack of TGF-β_1 may cause dysregulation of the production of immunosuppressive cytokines such as IL-4 and IL-10. Collectively, this may result in an uncontrollable proinflammatory cytokine cascade. Increased cytolytic functions of cytotoxic T cells, lymphokine-activated killer cells, and natural killer cells are also likely to be a contributing factor here since these functions are normally inhibited by TGF-β_1. In addition, the aberrant infiltration of inflammatory cells into tissues may result, in part, from loss of suppressive effects of TGF-β_1 on endothelial cell adhesiveness for neutrophils and lymphocytes (26, 27).

The trigger for the inflammatory response is unclear at present (autoimmune in nature or viral infection). Some of the inflammatory lesions (vasculitis, interstitial pneumonia, sialoadenitis) found in the TGF-β_1 (−/−) mice resembled those caused by murine pathogens such as pneumonia virus of mice, Kilham rat virus, minute virus of mice, and Sendai virus (25) and lesions found in immunological diseases (graft vs. host disease). Although there are several known murine viruses that cause minimal disease in normal mice (25), none of the lesions found in the TGF-β_1 (−/−) mice is pathognomonic for any known viral infection in normal mice. Graft-vs.-host reaction (GVHR) in mice produces lesions that resemble those in TGF-β_1 (−/−) mice, but they typically exhibit different patterns and occur in different organs (28–30). Common sites of GVHR lesions not found in TGF-β_1 (−/−) mice include renal glomeruli, thymus, liver, skin, and bone marrow. In an animal model of autoimmune disease, experimental autoimmune encephalomyelitis, systemic administration of antibodies specific for TGF-β_1 identified a role for endogenous TGF-β_1 in suppression of the disease (31). In this same model, immunohistochemical staining for TGF-β_1 has been demonstrated in foci of inflammatory brain lesions (32, 33). These findings suggest that the inability of TGF-β_1 (−/−) mice to express the growth factor may result in abnormal sensitivity to autoantigens and ultimately to the lethal phenotype observed.

TGF-β has been implicated in implantation of embryos in the uterus and all three isoforms are expressed in embryos as early as the four-cell stage (34–36). Our data suggest that an absence of TGF-β_1 in the embryos does not preclude implantation, although impairment of this function and possibly also

of other unknown functions of TGF-β_1 in the very early stages of embryogenesis may contribute to the lower birth-rate of TGF-β_1 (−/−) mice observed. It is also possible that TGF-β_2 and TGF-β_3 can substitute for some of these roles of TGF-β_1 early in embryogenesis (34) even though analysis of TGF-β_2 (data not shown) and TGF-β_3 mRNA levels in surviving TGF-β_1 (−/−) mice did not show enhanced expression. TGF-β_1 may also be important at later stages of gestation. The difference in survival may be influenced or determined by the precise genetic background of the TGF-β_1 (−/−) or their mothers due to genotype variations at loci other than TGF-β_1. At the present time it is not clear when and how the losses occur during intrauterine development.

In conclusion, the TGF-β_1 (−/−) mice represent a distinct phenotype and provide a valuable animal model to study the role of TGF-β_1 in various physiological and pathological processes such as inflammation, tissue repair, autoimmune disease, and allograft rejection (the latter two if cytokine or other treatment can lengthen their life span). Beyond these studies, the null mice could be used to study selective aspects of TGF-β function, such as the respective functions of each of the three mammalian isoforms, as could be done with replacement therapy with each of the isoforms. Furthermore, one can now consider the development of tissue-specific gene replacement therapy that would allow study of the physiology and pathology of TGF-β_1 in specific organs.

Note. A similar paper (37) was published while this paper was in the process of review.

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1. Roberts, A. B. & Sporn, M. B. (1990) in *Handbook of Experimental Pharmacology*, eds. Sporn, M. B. & Roberts, A. B. (Springer, Heidelberg), pp. 419–472.
2. Massagué, J. (1990) *Annu. Rev. Cell Biol.* **6**, 597–646.
3. Goey, H., Keller, J. R., Back, T., Longo, D. L., Ruscetti, F. W. & Wiltrout, R. H. (1989) *J. Immunol.* **143**, 877–880.
4. Bartlett, W. C., Purchio, A., Fell, H. P. & Noelle, R. J. (1991) *Lymphokine Cytokine Res.* **3**, 177–183.
5. Palladino, M. A., Morris, R. E., Starnes, H. F. & Levinson, A. D. (1991) *Ann. N.Y. Acad. Sci.* **593**, 181–187.
6. Lucas, C., Wallick, S., Fendly, B. M., Figuri, I. & Palladino, M. A. (1991) *Ciba Found. Symp.* **157**, 98–108.
7. Keller, J. R., Sing, G. K., Ellingsworth, L. R., Ruscetti, S. K. & Ruscetti, F. W. (1991) *Ann. N.Y. Acad. Sci.* **593**, 172–180.
8. Gresham, H. D., Ray, C. J. & O'Sullivan, F. X. (1991) *J. Immunol.* **146**, 3911–3921.
9. Wahl, S. M. (1992) *J. Clin. Immunol.* **12**, 61–74.
10. Capecchi, M. C. (1990) *Science* **244**, 1288–1292.
11. Robertson, E. J. (1991) *Biol. Reprod.* **44**, 238–245.
12. Geiser, A. G., Kim, S.-J., Roberts, A. B. & Sporn, M. B. (1991) *Mol. Cell. Biol.* **11**, 84–92.
13. McBurney, M. W., Sutherland, L. C., Adra, C. N., Leclair, B., Rudnicki, M. A. & Jardine, K. (1991) *Nucleic Acids Res.* **19**, 5755–5761.
14. Robertson, E. J. (1987) in *Teratocarcinoma and Embryonic Stem Cells: A Practical Approach*, ed. Robertson, E. J. (IRL, Oxford), pp. 71–112.
15. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
16. Tindall, K. R. & Stankowski, L. F., Jr. (1989) *Mutat. Res.* **220**, 241–253.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
18. Bradley, A. (1987) in *Teratocarcinoma and Embryonic Stem Cells: A Practical Approach*. Robertson, E. J. (IRL, Oxford), pp. 113–151.
19. Wakefield, L. M., Smith, D. M., Flanders, K. C. & Sporn, M. B. (1988) *J. Biol. Chem.* **263**, 7646–7654.
20. Flanders, K. C., Thompson, N. L., Cissel, D. S., van Obberghen-Schilling, E., Baker, C. C., Kass, M. E., Elingsworth, L. R., Roberts, A. & Sporn, M. B. (1989) *J. Cell. Biol.* **108**, 653–660.
21. Cherayil, B. J., Weiner, S. J. & Pillai, S. (1989) *J. Exp. Med.* **170**, 1959–1972.
22. Ward, J. M. & Sheldon, W. (1992) *Vet. Pathol.*, in press.
23. Mansour, S. L., Thomas, K. R. & Capecchi, M. R. (1988) *Nature (London)* **336**, 348–352.
24. Danielpour, D., Dart, L. L., Flanders, K. C., Roberts, A. B. & Sporn, M. B. (1989) *J. Cell. Physiol.* **138**, 79–86.
25. Lindsey, J. R., Boorman, G. A., Collino, M. J., Jr., Hsu, C. K., Van Hoosier, G. L. & Wagner, J. E. (1991) *Infectious diseases of mice and rats* (National Academy Press, Washington, DC).
26. Gamble, J. R. & Vadas, M. A. (1988) *Science* **242**, 97–99.
27. Gamble, J. R. & Vadas, M. A. (1991) *J. Immunol.* **146**, 1149–1154.
28. Rappaport, H., Khalil, A., Halle-Pannenko, O., Pritchard, L., Dantchev, D. & Mathé, G. (1979) *Am. J. Pathol.* **96**, 121–142.
29. Hart, M. N., Sadewasser, K. L., Cancilla, P. A. & DeBault, L. E. (1983) *Lab. Invest.* **48**, 419–427.
30. Staszak, C. & Harbeck, R. J. (1985) *Am. J. Pathol.* **120**, 99–105.
31. Miller, A., Lider, O., Roberts, A. B., Sporn, M. B. & Weiner, H. L. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 421–425.
32. Johns, L. D., Flanders, K. C., Ranges, G. E. & Sriram, S. (1991) *J. Immunol.* **147**, 1792–1796.
33. Racke, M. K., Cannella, B., Albert, P., Sporn, M., Raine, C. S. & McFarlin, D. E. (1992) *Immunology* **4**, 615–620.
34. Rappolee, D. A., Brenner, C. A., Schultz, R., Mark, D. & Werb, Z. (1988) *Science* **242**, 1823–1825.
35. Tamada, H., McMaster, M. T., Flanders, K. C., Andray, G. K. & Dey, S. K. (1990) *Mol. Endocrinol.* **4**, 965–972.
36. Paria, B. C., Jones, K. L., Flanders, K. C. & Dey, S. K. (1992) *Dev. Biol.* **151**, 91–104.
37. Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., Annunziata, N. & Doetschman, T. (1992) *Nature (London)* **359**, 693–699.