

Volume 12, issue 4, December 2001 | Articles originaux

Adenoviral supply of active transforming growth factor-beta1 (TGF-beta1) did not prevent lethality in transforming growth factor-beta1-knockout embryos

Abstract

In TGF-beta1-knockout mice, TGF-beta1-null conceptuses die during embryonic development with a penetrance of lethality that depends on the mouse genetic background. Studies have suggested that transplacental passage of maternal TGF-beta1 could account for the rescue of some TGF-beta1-null embryos.

Herein, we have used an adenovirus-based gene delivery system and a strain of mice where most TGF-beta1-null conceptuses die prior to parturition, to investigate whether an increase in maternal TGF-beta1 during pregnancy would rescue TGF-beta1-null embryos. A single intravenous injection of an adenovirus containing a modified version of TGF-beta1 cDNA (Ad-TGF-beta1S223/S225), coding for a biologically active form of the cytokine, induced a 20-fold increase in plasma TGF-beta1 (active and latent forms) levels for up to 3 months in adult mice. Similar levels of TGF-beta1 were detected in 13-day post coïtum (dpc) embryos from Ad-TGF-beta1-treated mothers, demonstrating an efficient maternal/fetal transfer of the cytokine. However, no increase in the frequencies of TGF-beta1-null neonates nor in day 11.5 dpc TGF-beta1-null conceptuses was observed despite elevated levels of TGF-beta1 delivered throughout gestation. In addition, we show that the high levels of TGF-beta1-titrated in the plasma from Ad-TGF-beta1S223/S225-treated mice were partly the consequence of a stimulation of an autocrine production by exogenous bioactive TGF-beta1. These results indicate that transplacental passage of TGF-beta1 was not effective in rescuing TGF-beta1-null conceptuses from embryonic lethality.

Full text

INTRODUCTION

Transforming growth factor-beta1 (TGF-beta1) is a ubiquitous cytokine belonging to a large family of structurally-related polypeptides involved in many cellular processes [1]. In the adult, TGF-beta1 is essentially a molecule that negatively regulates immune and inflammatory responses by inhibiting the proliferation of hematopoietic cells (for review see [2]). This function has been definitively demonstrated by lethality in TGF-beta1-knockout mice which succumb about 20 days after birth, from an acute wasting syndrome accompanied by an excessive infiltration of several organs by lymphocytes and macrophages [3, 4]. Biological actions of TGF-beta1 also include positive and negative regulation of extracellular matrix formation, osteogenesis, angiogenesis, cell differentiation and tumor progression [5, 6]. During embryogenesis, TGF-beta1 has been detected as early as the four-cell stage embryo [7, 8] and its role has been validated by the marked embryonic lethality in TGF-beta1-null embryos. The prenatal lethality is due to two different TGF-beta1 functions during embryogenesis: a requirement for pre-implantation development and an effect on yolk sac hemangioblasts, the precursor cells of

endothelial and hematopoietic lineages [9-11]. In TGF-beta1-knockout mice, it was noticed that the proportion of TGF-beta1-null embryos reaching parturition was markedly reduced, but that TGF-beta1-null neonates did not exhibit obvious developmental defects [3, 4]. This led to the hypothesis that some TGF-beta1-null embryos could be rescued by transplacental passage of maternal TGF-beta1 [12] or other members of the TGF-beta1 family. Subsequent reports have demonstrated that the extent of embryonic lethality was influenced by the genetic background of the intercrosses, suggesting a role of additional genetic factors. Using a genetic approach, a major codominant modifier gene for embryo lethality has been mapped on chromosome 5 [13]. The gene product is as yet unknown but it does not seem to act as a regulator of the level of maternal TGF-beta1 since lethality in TGF-beta1-null embryos could not be rescued by implantation into wild-type pseudopregnant females [10]. Thus, it seems likely that this gene may code either for a redundant molecule capable of replacing TGF-beta1 during embryogenesis or for a molecule which modifies the response to maternal TGF-beta1 [10].

TGF-beta1 is secreted by most cell types as part of a large multiprotein precursor complex in a biologically inactive form, called latent protein [14, 15]. *In vivo*, active TGF-beta1 is released from the complex by several proteins, including the multifunctional glycoprotein thrombospondin-1 [16, 17], plasmin [18] and some integrins [19]. Thus, conversion from the latent precursor molecule to the biologically active form is a key step in the regulation of TGF-beta1 biological activity. This activation occurs directly *in situ* but its precise regulation is still poorly understood. To determine if a defect in the activation of maternal TGF-beta1 could explain lethality in TGF-beta1-null embryos, we injected a recombinant adenovirus encoding for an active form of TGF-beta1 into heterozygote TGF-beta1 females which were mated with heterozygote TGF-beta1 male mice and we studied the frequency of birth of TGF-beta1-null embryos. Despite the presence of elevated levels of active TGF-beta1 in the pregnant females and in the embryos, the lethality in TGF-beta1-null embryos was not modified. This result suggests that the product of the genetic modifier of embryonic lethality does not appear to be a molecule acting on the activation of TGF-beta1 and may more likely be a redundant molecule for endothelial cell differentiation.

MATERIALS AND METHODS

Animals

Heterozygous TGF-beta1 mice with a C57Bl/6 genetic background were kindly received from Dr. A. Kulkarni (NIH, Bethesda, MD, USA) [4]. Control C57Bl/6J mice were purchased from Iffa Credo (L'Arbresle, France). Mice were bred at the Institut Gustave Roussy animal facilities under specific pathogen-free conditions. Mice injected with the adenovirus vectors were kept in a negatively pressurized isolator as recommended.

Genotyping

Tail tips from 10 day-old pups were digested overnight at 56° C in 0.5 ml of lysis buffer (50 mM Tris pH8, 20 mM EDTA, 0.5% SDS, 150 mM NaCl) plus 1 mg/ml of proteinase K. DNA was phenol/chloroform extracted and precipitated in 100% ethanol. An aliquot of DNA (500 ng) was used in a 50 mul polymerase chain reaction (PCR) to amplify either the normal TGF-beta1 allele or the mutated one. To amplify the normal allele, oligonucleotides were derived from the deleted portion of exon 1 5'-GGTCACCCGCGTGCTAATGG-3' and from intron 1 5'-GAGAGTAAGCCCACTAGAG-3' to give a 529 base pair product. To amplify the targeted allele, oligonucleotides were derived from exon 1 5'-ATGGAGCTGGTGAAACGGAA-3' and from the phosphoglycerate kinase promoter 5'-TCCATCTGCACGAGACTAGT-3' to give a product size of 375 base pairs. The reaction mixture was amplified by 35 cycles as follows: denaturation for 5 min at 94° C, annealing for 30 sec at 55° C and extension at 72° C for 30 sec in a thermocycler 2,400 (Perkin Elmer Co, Courtab▒uf, France). PCR products were loaded on 2% agarose gel and visualized by ethidium bromide staining.

*Adenovirus encoding biologically active TGF-*beta*1*

The recombinant adenovirus encoding TGF-beta1, a kind gift from Dr. P.. Moullier (Nantes, France), has been described previously [20]. Briefly, the El and E3 regions of the adenovirus vector were deleted and carried a thermosensitive mutation in the E2a region. Cysteine residues located at amino acid positions 223 and 225 in the pro region of the TGF-beta1 precursor were changed to a Ser codon by site-directed mutagenesis resulting in the secretion of biologically active TGF-beta1 [21]. The mutated cDNA was placed under the transcriptional control of the CMV promoter (Ad-TGF-beta1$^{S223/S225}$). An empty vector was used

as a control. Recombinant adenoviruses were titered on 293 cells using plaque-forming unit (pfu) assays and are expressed as pfu/ml.

*Determination of TGF-*beta*1-level*

Blood was collected on EDTA anticoagulant. Plasma was prepared by 2 successive centrifugations (5 min, 5,000 rpm at room temp) and stored at - 20° C until use. Embryos were individually collected in an eppendorf tube and centrifuged for 10 min at 13,000 rpm to extract the fluid contained in the bodies. Fluids were stored at - 20° C until use. An enzyme linked immunosorbent assay (ELISA) kit (TGF-beta1 Emax TM Immunoassay System, Promega) which detects only the active form of TGF-beta1 was used accordingly to the manufacturer's recommendations. Levels of TGF-beta1 were determined before (only the spontaneously active form) and after acidification (active and latent forms). For acidification, 1 mul of 1N HCL was added to 50 mul diluted samples (1:5 in Dulbecco's phosphate-buffered saline (DPBS, Gibco); samples were incubated at room temperature for 15 min and neutralized by addition of 1 mul of 1N NaOH. The sensitivity of the ELISA was 32 pg/ml of active TGF-beta1.

*Culture of embryo fibroblasts and Ad-TGF-*beta*1-infection*

Primary fibroblast cultures were derived from day-13 conceptuses obtained from heterozygote intercrosses. Part of the embryo body was used to prepare DNA for PCR genotyping. Cell suspensions were prepared by trypsinization. Cells (2 x 10^5/ml) were seeded in 12-well plates in 2 ml alpha minimal essential medium (alpha-MEM, Gibco, BRL) supplemented with penicillin, streptomycin and 10% fetal bovine serum (FBS). When cells reached semi-confluence, they were infected with 50 pfu/cell of Ad-TGF-beta1$^{S223/S225}$ or mock vector over 24 hours, after which time the medium was changed and replaced by alpha-MEM containing 2% FBS. Infected cells were incubated for an additional 48 hours and 96 hours prior to supernatant collection.

RESULTS

*TGF-*beta*1-production in Ad-TGF-*beta*1$^{S223/S225}$-treated mice*

To assess TGF-beta1 production, a dose-response study was first performed in adult C57BL/6 mice. Groups of 15 mice were injected intravenously with different

doses of Ad-TGF-beta1$^{S223/S225}$. At time points indicated (Figure 1), the plasma from 3 mice were assayed by ELISA after acidification to monitor the increase in level and the duration of TGF-beta1 production. At the dose of 1 x 10^9 pfu of virus, none of the mice survived beyond 5 days. Lower doses were not detrimental to the animals and led to a 4- to 7-fold increase in plasma TGF-beta1 that persisted for several weeks. A study was next performed with adult heterozygote TGF-beta1 mice, which revealed that the same doses of Ad-TGF-beta1$^{S223/S225}$ yielded similar results (data not shown). In 10 day-old pups injected intraperitoneally, the maximal non-lethal dose was 2 x 10^6 pfu per mouse. Fifteen days after injection, blood samples were collected and levels of TGF-beta1 were determined prior to and after acidification. As illustrated in Figure 2, no active TGF-beta1 was detected in the plasma from untreated controls, while 2.5 ± 0.5 ng/ml of TGF-beta1 was detected after acidification (n = 5). In contrast, active TGF-beta1 was readily detectable at a mean concentration of 3 ng/ml (range = 1.6-7 ng/ml) in non-acidified plasma from Ad-TGF-beta1-treated pups (n = 5) and the total amount of TGF-beta1 (latent + active forms) was found to have increased 12-fold. The elevation in plasma TGF-beta1 levels was related to the transgene expression since no increase in TGF-beta1 levels was seen in plasma from mice infected with 2 x 10^6 pfu of empty Ad-vector (Figure 2). These data indicate that injection of mice with a recombinant adenovirus encoding the mutated form of TGF-beta1 resulted in the detection of active TGF-beta1 in the circulation, but also in a massive augmentation of latent TGF-beta1.

*TGF-*beta*1-is transferred from mother to fetuses*

To assess whether maternal TGF-beta1 derived from the recombinant adenovirus would cross the maternal/fetal barrier and diffuse into the fetuses, females were i.v. injected with 2 x 10^8 pfu of virus 48 hours before mating. Animals were sacrificed on day 13 *post coïtum* and embryos were harvested. Plasma from mothers and fluids prepared from dissected individual embryos were tested for the presence of TGF-beta1 by ELISA prior to and after acidification. Without acidification of the samples, active TGF-beta1 was detected in mothers' plasma (3 ± 1 ng/ml; n = 8) and embryo fluids (2.2 ± 0.6 ng/ml; n = 17) at similar levels (Figure 3). In addition, the levels of latent TGF-beta1 were highly elevated in mothers' plasma (45.2 ± 8.3 ng/ml), as well as in embryos fluids (36.4 ± 6.8 ng/ml). To ensure that the presence of active TGF-beta1 in embryo fluids was not the result of an activation of latent TGF-beta1 during the preparation process, experiments were carried out with embryos

harvested from untreated mothers. In every sample (n = 8), active TGF-beta1 was detectable but at a mean concentration of 460 pg/ml (range = 270-700 pg/ml) which was 3- to 8-fold lower than in embryos taken from treated mothers. In addition, the levels of total TGF-beta1 in these samples never exceeded 6 ng/ml, a measurement in sharp contrast to the mean concentration of 36 ng/ml found in embryos taken from mothers injected with Ad-TGF-beta1. Together, these data indicate that the protein encoded by adenovirus vector crossed the placental barrier and diffused into the fetuses.

*Maternal transfer of active TGF-*beta*1 did not reduce embryonic lethality in TGF-*beta*1-null embryos*

To analyze whether a continuous delivery of active TGF-beta1 during pregnancy would improve the frequency of birth of TGF-beta1-null pups, heterozygote females were injected with 2×10^8 pfu of Ad-TGF-beta1$^{S223/S225}$ before mating. Overexpression of TGF-beta1 did not impair fertility since no significant difference was seen in the number of litters or neonates per litter (5 to 7 pups/litter). PCR was used to analyze the genotypic frequency of the progeny. The results (Table 1) show that the frequency of birth of TGF-beta1-null pups was not improved since the ratio in each genotype class remained unchanged as compared to untreated mothers (< 1% TGF-beta1-null pups). Since TGF-beta1 is required for yolk sac hematopoiesis and endothelial differentiation [9-11], we next asked whether delivery of active TGF-beta1 would improve the development of TGF-beta1-null conceptuses. Genotype analysis was performed on 11.5 dpc conceptuses from Ad-TGF-beta1-treated heterozygote or untreated females intercrossed with heterozygote males. Out of 60 conceptuses from Ad-TGF-beta1-treated mothers analyzed, 18 were wild-type, 40 were heterozygote and 2 were homozygote. A comparable proportion was seen in embryos from untreated mothers. Together, these results indicate that transplacental passage of biologically active TGF-beta1 was ineffective at rescuing TGF-beta1-null conceptuses from embryonic lethality.

*Autocrine stimulation of TGF-*beta*1-synthesis by Ad-TGF-*beta*1$^{S223/S225}$*

In mice injected with the Ad-TGF-beta1$^{S223/S225}$, a dramatic elevation in the plasma levels of latent TGF-beta1 was observed suggesting that the active protein could modulate its own synthesis in the body. To tackle this question, embryonic fibroblasts from wild-type or TGF-beta1-null embryos were infected

with 50 pfu of Ad-TGF-beta1$^{S223/S225}$/cell or empty vector. Uninfected cultures served as controls. Culture supernatants were collected at 48 hours and 96 hours and concentrations of active and latent TGF-beta1 were determined before and after acidification (Figure 4). TGF-beta1-was barely detectable in acidified supernatants from control, uninfected cultures or in cultures infected with the empty adenovirus vector. In contrast, active TGF-beta1 was readily detectable at similar levels (0.2 ng/ml) in 96 hours post-infection supernatants from wild-type and TGF-beta1-deficient cultures. Following acid treatment, the mean level of TGF-beta1 was increased 12-fold (3 ± 1 ng/ml) in supernatants from wild-type cultures, whereas only a 5-fold increase (1.3 ± 0.6 ng/ml) was measured in TGF-beta1-deficient cultures. Since no endogenous TGF-beta1 is synthesized by TGF-beta1-null fibroblasts, the total amount of TGF-beta1 found in supernatants represents the synthesis yielded by the vector. In wild-type cultures, 3-fold greater activity was reproducibly observed. These observations are thus consistent with a potent autostimulatory effect of the biologically active protein on TGF-beta1 synthesis.

DISCUSSION

In this report, we evaluated the use of an adenovirus-based delivery of bioactive TGF-beta1 to rescue TGF-beta1-null conceptuses from prenatal lethality. Data presented here demonstrate that treatment of mice with a single intravenous injection of 2×10^8 pfu of Ad-TGF-beta1$^{S223/S225}$ led to a potent and long-lasting expression of the transgene, resulting in a 20-fold increase in plasma TGF-beta1 (active and latent forms) and an efficient transplacental transfer of the cytokine from mothers to fetuses. However, no rescue of TGF-beta1-null conceptuses was observed.

Studies with TGF-beta1-knockout mice have revealed the important role played by TGF-beta1 during embryogenesis leading to more or less penetrant lethality in TGF-beta1-null embryos [9-11, 13]. Furthermore, it has been demonstrated that the penetrance of the lethal phenotype is directly influenced by the genetic background of the intercrosses, with 50% lethality in certain strains of mice to nearly complete lethality in the C57Bl/6 background [9, 10, 13]. To explain the variable penetrance of the lethal phenotype of the TGF-beta1-null embryos, it has been suggested that varying levels of transplacental transfer of maternal TGF-beta1 or other TGF-betas could account for the rescue of some null embryos during embryogenesis [3, 9, 12]. However, this hypothesis was ruled out

by embryo transfer experiments showing that transfer of two-cell conceptuses obtained from heterozygote TGF-beta1 crosses into pseudogestant wild-type mothers did not reduce the degree of TGF-beta1-null embryonic lethality [10]. Our data are in accordance with the results from Kallapur *et al.* [10], and further support the notion that maternal TGF-beta1 does not play a significant role in rescuing TGF-beta1-null conceptuses. Indeed, we show that treatment of mice with an adenovirus encoding a biologically active form of TGF-beta1 resulted in a massive increment in maternal plasma TGF-beta1 and in an efficient maternal/embryo transfer throughout gestation. Nevertheless, the frequency of birth of TGF-beta1-null pups was not increased. Furthermore, despite the high levels of bioactive TGF-beta1 in maternal plasma at conception and during gestation, no increase in 11.5 dpc TGF-beta1-null embryos was detected, indicating that the exogenous TGF-beta1 delivered from the vector was not efficient in rescuing embryos from intrauterine death. During murine embryogenesis, TGF-beta1 transcription and protein synthesis have been detected at the four-cell stage suggesting an important role of the cytokine throughout development [7, 22-24]. At the level of hematopoietic stem cells, evidence has been provided that TGF-beta1 controls quiescence, in part through an autocrine loop [25-27]. Thus, it cannot be excluded that different biological effects might be elicited from autocrine *versus* humoral TGF-beta1 synthesis. Alternatively, active TGF-beta1 has the ability to down-modulate the expression of several hematopoietic cytokine receptors [28-31]. Since the plasma level of bioactive TGF-beta1 in Ad-TGF-beta1$^{S223/S225}$-treated mice was quite high (1-2 ng/ml), we cannot exclude some possible indirect regulatory effects.

The existence of genetic modifiers which profoundly influence the phenotype of mutant mice has been established in studies with other gene disruption models [32-34]. In the TGF-beta1-knockout mice, a major dominant modifier locus, which modulates the function of TGF-beta1, has been identified and mapped to proximal mouse chromosome 5 [13]. Although, the gene and its product have not yet been identified, it was speculated that the modifier gene product could act by allowing embryos to respond to limited supplies of maternal TGF-beta1 or to other members of the TGF-beta family or to proteins that might increase TGF-beta1 activity or signalling. In this study, we have used an adenovirus vector carrying a mutated TGF-beta1 cDNA which was previously shown to encode about 70% of spontaneously bioactive forms [21]. Despite the presence of active TGF-beta1 protein in the treated mothers and fetuses, the frequency of birth of TGF-beta1-null pups did not increase. Hence, the modifier gene

modulating lethality in TGF-beta1-null embryos does not appear to be involved in the process of TGF-beta1 activation. Furthermore, our data also suggest that the product of the modifier gene does not code for a molecule directly involved in TGF-beta1 signaling because lethality persists despite high TGF-beta levels. It seems more likely that this gene may encode for a cytokine redundant with TGF-beta1 during embryogenesis.

It is worth noting that an overexpression of latent TGF-beta1 was found both in plasma from mice treated with the Ad-TGF-beta1$^{S223/S225}$ as well as in embryo bodies, suggesting that the bioactive adenovirus-encoded form of TGF-beta1 could up-regulate its own synthesis [35, 36]. To clarify this point, we used primary cultures of fibroblasts from wild-type and TGF-beta1-deficient embryos which were infected with the Ad-TGF-beta1$^{S223/S225}$ vector. As expected, similar levels of spontaneously active protein were detected in supernatants from both types of culture (Figure 4). However, when supernatants from TGF-beta1-null cultures were tested after acidification, levels of activity increased 5-fold, demonstrating that only 20% of the protein was secreted in an active form. These results are not consistent with previous data indicating that TGF-beta1$^{S223/S225}$ was at least 70% active without acid treatment [21]. Sequencing of the mutated region confirmed the presence of the 2 expected amino acids changes and did not reveal other mutations. It is most likely that the different cell types or the different strategies used in the two studies might account for this discrepancy. Nevertheless, TGF-beta1 titrated in supernatants from wild-type cultures was about 3 times higher than in supernatants from TGF-beta1-deficient fibroblasts, strongly suggesting an autocrine up-regulation.

CONCLUSION

In conclusion, this study demonstrates that the adenovirus-mediated TGF-beta1 delivery was an efficient strategy allowing a potent expression of TGF-beta1 during at least 3 months and without detrimental effects on the animals. However, despite transplacental transfer of both bioactive and latent forms, lethality in TGF-beta1-null embryos was not improved. This suggests that an intracrine stimulation by TGF-beta1 might be crucial for embryonic development. Further experiments will be required to pursue this hypothesis.

Acknowledgements. The authors gratefully acknowledge Dr. A. Kulkarni for the TGF-beta1-knockout mice and P. Moullier for providing the adenoviral

supernatants. We also thank the SCEA at the Institut Gustave-Roussy for help with animal husbandry. This work was supported by INSERM, IGR and la Ligue Nationale contre le Cancer (équipe labellisée). H.C. was a recipient of a fellowship from the French Ministry of Science and Technology.

References

1. Sporn M B, Roberts A B. 1991. Introduction: what is TGF-beta? Clinical application of TGF-beta. (Review) *Ciba Found Symp.* 157: 1.

2. Bottinger E P, Letterio J J, Roberts A B. 1997. TGF-beta knockout and transgenic mouse models. (Review) *Kidney Int.* 51: 1355.

3. Shull M M, Ormsby I, Kier A, Pawlowski S, Diebold R J, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Annunziata N, Doetschman T. 1992. Targeted disruption of the mouse transforming growth factor-beta1 gene results in multifocal inflammatory disease. *Nature* 359: 693.

4. Kulkarni A B, Huh C G, Becker D, Geiser A, Lyght M, Flanders K C, Roberts A B, Sporn M B, Ward J S, Karlsson S. 1993. Transforming growth factor-beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc. Natl. Acad. Sci. USA* 90: 770.

5. Massagué J. 1990. The transforming growth factor-beta family. (Review) *Annu. Rev. Cell Biol.* 6: 597.

6. Roberts A B, Sporn M B. 1990. The transforming growth factor-betas. In: Sporn M B, Roberts A B. Peptide growth factors and their receptors. New York Inc: Springer-Verlag, 419.

7. Rappolee D A, Brenner C A, Schultz R, Mark D, Werb Z. 1988. Developmental expression of PDGF, TGF-alpha and TGF-beta genes in preimplantation mouse embryos. *Science* 241: 1823.

8. Pelton R, Saxena B, Jones M, Moses H L, Gold L L. 1991. Immunohistochemical localization of TGF-beta1, TGF-beta2 and TGF-beta3 in the mouse embryo:

expression patterns suggest multiple roles during embryonic development. *J. Cell Biol.* 115: 1091.

9. Dickson M C, Martin J S, Cousins F M, Kulkarni A B, Karlsson S, Akhurst R J. 1995. Defective haematopoiesis and vasculogenesis in transforming growth factor-beta1 knockout mice. *Development* 121: 1845.

10. Kallapur S, Ormsby I, Doetschman T. 1999. Strain dependency of TGF-beta1 function during embryogenesis. *Mol. Reprod. Dev.* 52: 341.

11. Larsson J, Goumans M J, Sjöstrand L J, van Rooijen M A, Ward D, Levéen P, Xu X, ten Dijke P, Mummery C L, Karlsson S. 2001. Abnormal angiogenesis but intact hematopoietic potential in TGF-beta1 type I receptor-deficient mice. *EMBO J.* 20: 1663.

12. Letterio J J, Gieser A G, Kulkarni A B, Roche N S, Sporn M B, Roberts A B. 1994. Maternal rescue of the transforming growth factor-beta1 null mice. *Science* 264: 1936.

13. Bonyadi M, Rusholme S A, Cousins F M, Su H C, Biron C A, Farrall M, Akhurst R J. 1997. Mapping of a major genetic modifier of embryonic lethality in TGF-beta1-knockout mice. *Nat. Genet.* 15: 207.

14. Wakefield L M, Smith D M, Flanders K C, Sporn M B. 1988. Latent transforming growth factor-beta from human platelets. A high molecular weight complex containing precursor sequences. *J. Biol. Chem.* 263: 7646.

15. Miyazono K, Hellman U, Werstedt C, Heldin C H. 1988. Latent high molecular weight complex of trasforming growth factor-beta1. Purification from human platelets and structural characterization. *J. Biol. Chem.* 263: 6407.

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

17. Crawford S E, Stellmach V, Murphy-Ullrich J E, Ribiero S M, Lawler J, Hynes R O, Boivin G P, Bouck N. 1998. Thrombospondin-1 is a major activator of TGF-beta1 *in*

vivo. Cell 93: 1159.

18. Lyons R M, Gentry L E, Purchio A F, Mosses H L. 1990. Mechanism of activation of latent recombinant transforming growth factor-beta1 by plasmin. *J. Cell Biol.* 110: 1361.

19. Munger J S, Huang X, Kawakatsu H, Griffiths M J, Dalton S L, Wu J, Pittet J F, Kaminski N, Garat C, Mattlay M A, Rifkin D B, Sheppard D. 1999. The integrin alpha5beta6 binds and activates latent TGF-beta1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 96: 319.

20. Josien R, Douillard P, Guillot C, Müschen M, Anegon I, Chetritt J, Menoret S, Vignes C, Soulillou J P, Cuturi M C. 1998. A critical role for transforming growth factor-beta in donor transfusion-induced allograft tolerance. *J. Clin. Invest.* 102: 1.

21. Brunner A M, Marquardt H, Malacko A R, Lioubin M N, Purchio A F. 1989. Site-directed mutagenesis of cysteine residues in the pro region of the transforming growth factor beta1 precursor. Expression and characterization of mutant protein *J. Biol. Chem.* 264: 13660.

22. Heine U, Munoz E F, Flanders K C, Ellingsworth L R, Lam H Y, Thompson N L, Roberts A B, Sporn M B. 1987. Role of transforming growth factor-beta in the development of the mouse embryo. *J. Cell Biol.* 105: 2861.

23. Akhurst R J, Lehnert S A, Faissner A, Duffie E. 1990. TGF-beta in murine morphogenetic processes: the early embryo and cardiogenesis. *Development* 108: 645.

24. Paria B C, Jones K L, Flanders K C, Dey S K. 1992. Localisation and binding of transforming growth factor-beta isoforms in mouse preimplantation embryos and in delayed and activated blastocysts. *Dev. Biol.* 151: 91.

25. Hatzfeld J, Li M L, Brown E L, Sookdeo H, Levesque J P, O'Toole T, Gurney C, Clark S C, Hatzfefd A. 1991. Release of early human hematopoietic progenitors from quiescence by antisense transforming growth factor-beta1 and Rb oligonucleotides. *J. Exp. Med.* 174: 925.

26. Ploemacher R E, van Soest P L, Boudewijn A. 1993. Autocrine transforming growth factor-beta1 blocks colony formation and progenitor cell generation by hematopoietic stem cells stimulated with steel factor. *Stem. Cells* 11: 336.

27. Fortunel N O, Hatzfeld A, Hatzfeld J A. 2000. Transforming growth factor-beta: pleiotropic role in the regulation of hematopoiesis. (Review) *Blood* 96: 2022.

28. Dubois C M, Ruscetti F W, Palaszynski E W, Falk L A, Oppenheim J J, Keller J R. 1990. Transforming growth factor-beta is a potent inhibitor of interleukin 1 (IL-1) receptor expression: proposed mechanism of inhibition of IL-1 action. *J. Exp. Med.* 172: 737.

29. Jacobsen S E, Ruscetti F W, Dubois C M, Lee J, Boone T C, Keller J R. 1991. Transforming growth factor-beta trans-modulates the expression of colony stimulating factor receptors on murine hematopoietic cell lines. *Blood* 77: 1706.

30. Dubois C M, Ruscetti F W, Stankova J, Keller J R. 1994. Transforming growth factor-beta regulates c-kit message stability and cell-surface protein expression in hematopoietic progenitors. *Blood* 83: 3138.

31. Heinrich M C, Dooley D C, Keeble W W. 1995. Transforming growth factor-beta1 inhibits expression of the gene products fior steel factor and its receptor (c-kit). *Blood* 85: 1769.

32. Threadgill D W, Dlugozl A A, Hansen L A, Tennenbaum T, Lichti U, Yee D, LaMantia C, Moutron T, Herrup K, Harris R C, *et al.* 1995. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* 269: 230.

33. Liu J P, Baker J, Perkins A S, Robertson E J, Efstradiatis A. 1993. Mice carrying null mutations of the genes encoding insulin-like growth factor I *(Igf-1)* and type 1 IGF receptor *(Igf1r). Cell* 75: 59.

34. Rozmahel R, Wilschanski M, Matin A, Plyte S, Oliver M, Auerbach W, Moore A, Forstner J, Durie P, Nadeau J, Bear C, Tsui L C. 1996. Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. *Nat. Genet.* 12: 280.

35. Blanchette F, Day R, Dong W, Laprise M H, Dubois C M. 1997. TGF-beta1 regulates gene expression of its own converting enzyme furin. *J. Clin. Invest.* 99: 1974.

36. Kim S J, Jeang K T, Glick A B, Sporn M B, Roberts A B. 1989. Promoter sequences of the human transforming growth factor-beta1 gene responsive to transforming growth factor-beta1 autoinduction. *J. Biol. Chem.* 264: 7041.

Figures

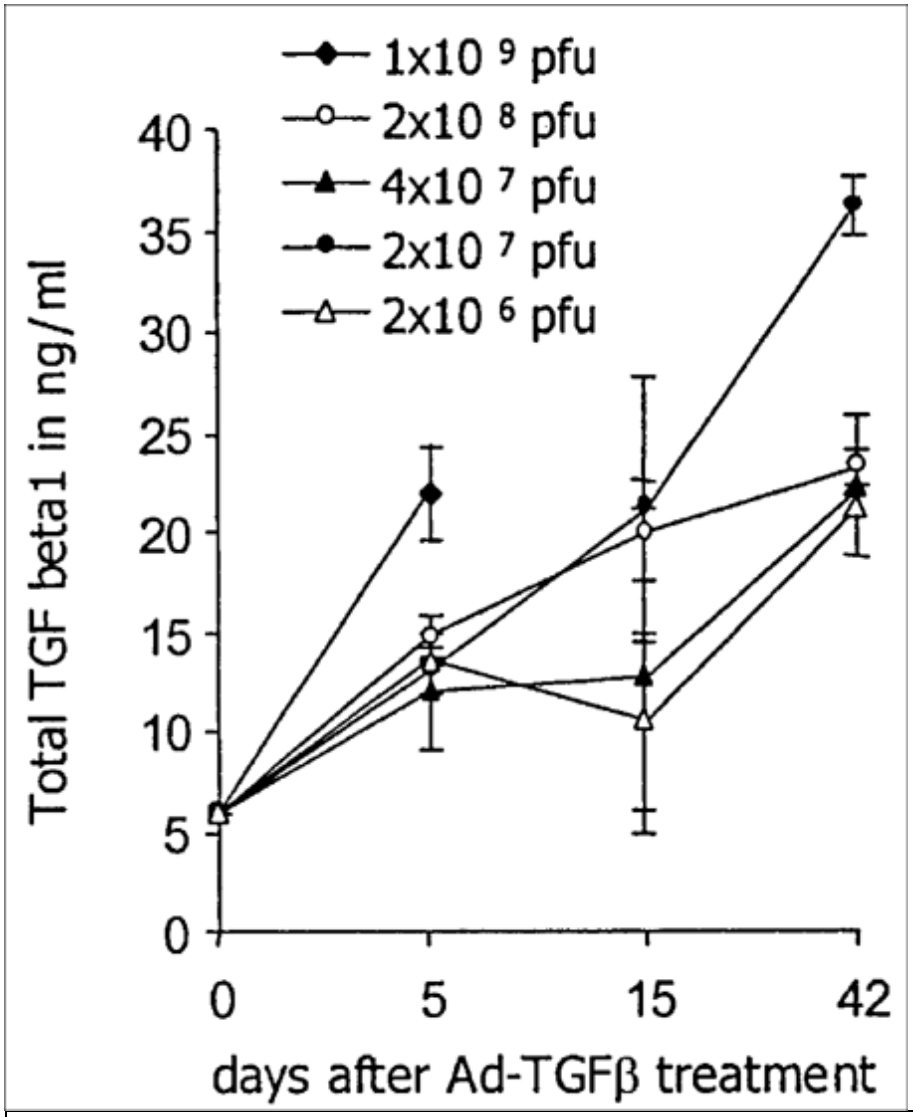


Figure 1
TGF-beta1 quantification in plasma from mice injected with Ad-TGF-beta1$^{S223/S225}$. Mice were intravenously injected with varying doses of recombinant Ad-TGF-beta1$^{S223/S225}$ (1×10^9 to 2×10^6 pfu/mouse). Plasma from 3

mice was collected at the times indicated. An enzyme-linked immunosorbent assay (ELISA) was used to quantify TGF-beta1 afte

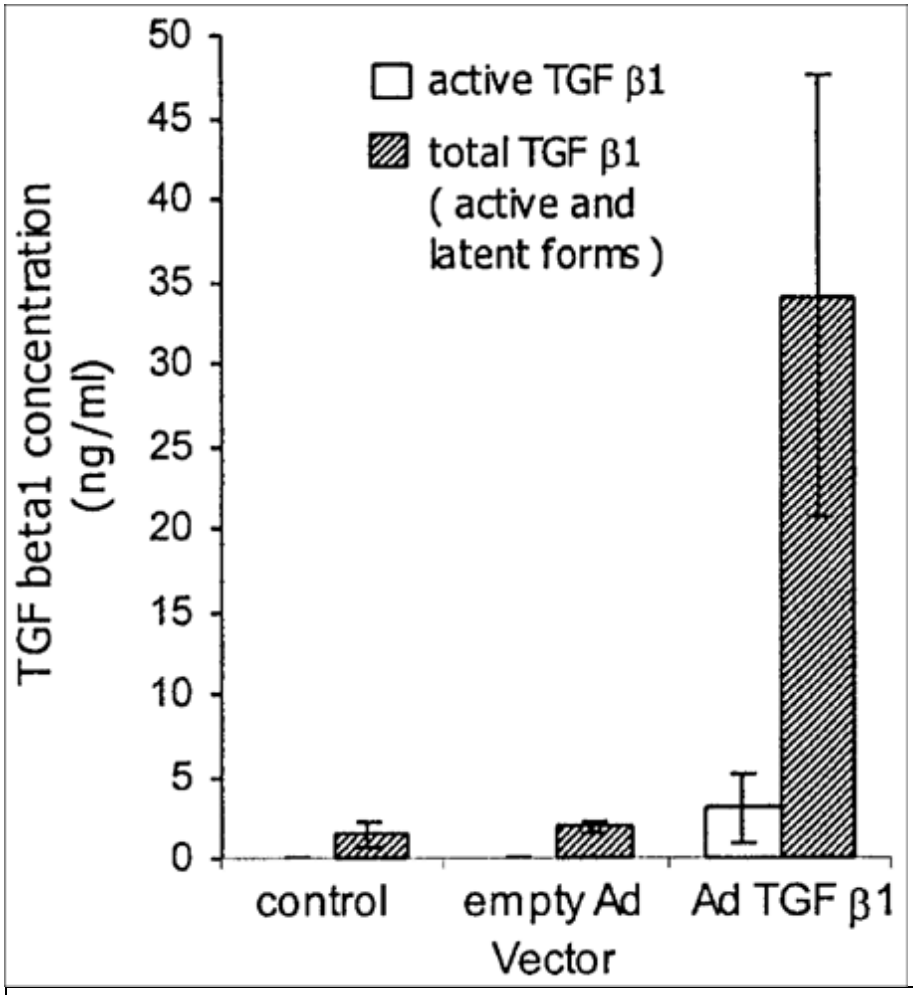


Figure 2

TGF-beta1 quantification in plasma from young mice injected with Ad-TGF-beta1$^{S223/S225}$. Ten day-old pups were injected with 2 x 10^6 pfu of Ad-TGF-beta1$^{S223/S225}$ (n = 8) or the empty adenovirus vector (n = 5). ELISA was used to quantify active TGF-beta1 (open bars) or total (active and latent forms) after samples acidification (hatched bars). Control group comprises untreated animals (n = 5). Data are expressed as mean ± SD.

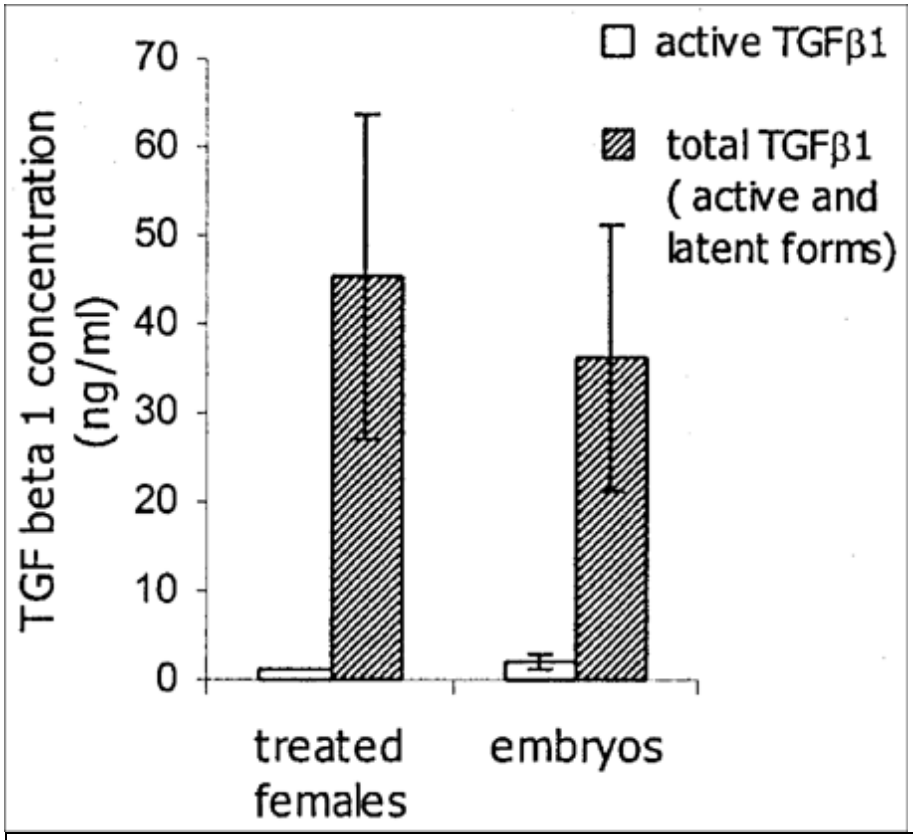


Figure 3

Active and latent TGF-beta1 diffuses from mothers to fetuses. Heterozygote females (n = 8) were injected 2 days before mating with 2 x 10^8 pfu of Ad-TGF-beta1$^{S223/S225}$. Females were sacrificed at day 13 *post coitum* and embryos were harvested. Levels of TGF-beta1 in plasma from treated mothers (about 2 months post-adenovirus injection) and embryo fluids (n = 17) were analyzed by ELISA without (active) or with acidification (active + latent). Open bars represent active TGF-beta1 and hatched bars represent total (active + latent) TGF-beta1. Data are expressed as the mean ± SD.

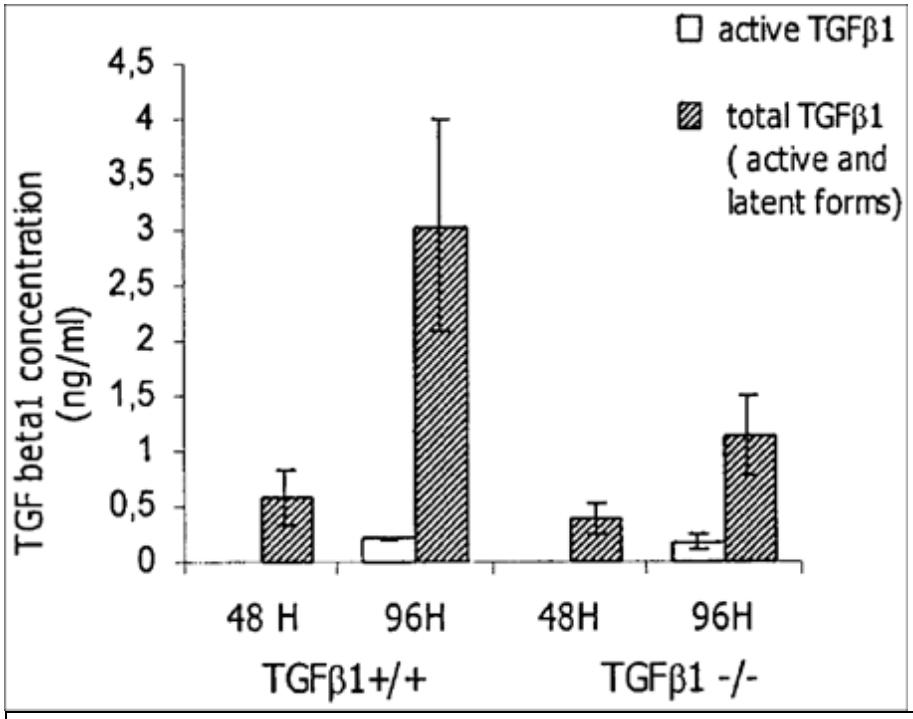


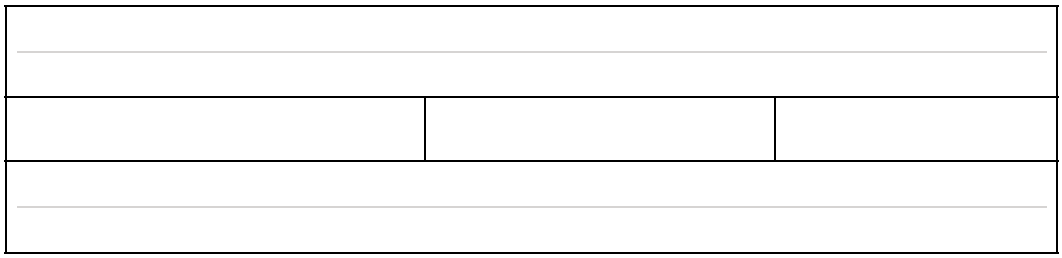
Figure 4

Autocrine production of latent TGF-beta1 in cultures infected with Ad-TGF-beta1$^{S223/S225}$. Embryonic fibroblasts from wild-type and TGF-beta1-null embryos were infected with 50 pfu/cells of Ad-TGF-beta1$^{S223/S225}$ or empty vector. Culture supernatants were collected at times indicated and assayed with an ELISA prior to (open bars) and after acidification of the samples (hatched bars). Data are expressed as the mean ± SD of 3 repeated experiments performed in duplicate.

Table 1
Genotypes of pups born from Ad-TGF-β1$^{S223/S225}$-treated or untreated mothers

Treatment of mothers	Genotypes TGF-β1 +/+	TGF-β1 +/–	TGF-β1 –/–	Number of pups analysed
With Ad-TGF-β1	32%	67%	1%	107
Without Ad-TGF-β1	34%	65%	1%	347

Heterozygote TGF-β1 males were mated with heterozygote females pre-treated with Ad-TGF-β1$^{S223/S225}$ or untreated. The genotype of pups was determined by PCR. Sixteen littermates born from Ad-TGF-β1 pre-treated mothers were examined.



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Recombinant human interleukin-1 beta: new possibilities for the prophylaxis and correction of toxic myelodepression in patients with malignant tumors I. Phase I-II clinical trials of recombinant human interleukin-1 beta as a leukopoiesis stimulator in cancer patients receiving combination chemotherapy

M.L. Gershanovich, L.V. Filatova, S.A. Ketlinsky, A.S. Simbirtsev
human recombinant interleukin-1 beta, myelopoiesis, myelodepression, cancer chemotherapy.

Recombinant human interleukin-1 beta: new possibilities for the prophylaxis and correction of toxic myelodepression in patients with malignant tumors II.

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