16. Y. Choi *et al., Proc. Natl. Acad. Sci. U.S.A.* **86**, 8941 (1989); A. M. Pullen *et al., Cell* **61**, 1365 (1990).
17. Northern (RNA) blot analysis of transcripts encoded by the defective virus genome in B6-1710, B6-1153, and B6xCBA/N-2252 were performed with polyadenylated [poly(A)$^+$] mRNA hybridized with a ^{32}P-labeled probe for the defective virus genome. Transcript levels for B6-1710 and B6xCBA/N-2252 were approximately tenfold higher than for B6-1153. Immunoblot analyses of *gag*-encoded proteins in the cytoplasm and on the surface of B6-1710 and B6-1153 were performed with goat antisera to MuLV p12 and p30 (Program and Logistics, Viral Oncology, National Cancer Institute, Bethesda, MD) or anti-p12 and anti-p30 (Fig. 1, legend). For B6-1710, anti-p30 and both antisera revealed the presence of 60- and 65-kD proteins in both locations; anti-p12 differed in failing to react with a 60-kD protein on the surface of these cells. For B6-1153, a 60-kD cytoplasmic but not surface protein was detected with the antisera but the monoclonal antibodies did not detect proteins from either source (S. K. Chattopadhyay, S. K. Ruscetti, H. C. Morse III, unpublished observations).
18. A. W. Hügin and H. C. Morse III, unpublished observations.
19. D. Woodland *et al., Science* **247**, 964 (1990); N.-S. Liao *et al., J. Immunol.* **144**, 844 (1990).
20. J. Bill *et al., J. Mol. Cell. Immunol.* **4**, 269 (1990).
21. A. Cerny *et al.*, in preparation.
22. K. B. Cease *et al., Proc. Natl. Acad. Sci. U.S.A.* **84**, 4249 (1987); M. P. N. Nair *et al., ibid.* **85**, 6498 (1988); J. A. Berzofsky *et al., Nature* **334**, 706 (1988); R. F. Siciliano *et al., J. Immunol.* **142**, 1506 (1989); R. D. Schrier *et al., ibid.*, p. 1166; S. Abrignani *et al., Proc. Natl. Acad. Sci. U.S.A.* **87**, 6136 (1990); J. Krowka *et al., J. Immunol.* **144**, 2535 (1990).
23. H. Golding *et al., J. Exp. Med.* **167**, 914 (1988).
24. B. Chesebro *et al., Virology* **127**, 134 (1983).
25. Unlabeled monoclonal antibodies with specificity for $V_\beta3$ [KJ25 (*14*)]; $V_\beta5.1$ and $V_\beta5.2$ [MR9-4; E. P. Reich *et al., Nature* **341**, 326 (1989)]; $V_\beta5.2$ only [MR9-8 (*19*)]; $V_\beta6$ [RR4-7; O. Kanagawa *et al., Cell. Immunol.* **119**, 412 (1989)]; $V_\beta7$ [TR-310; C. Y. Okada and I. L. Weissman, *J. Exp. Med.* **169**, 1703 (1990)]; $V_\beta8.1$, $V_\beta8.2$, and $V_\beta8.3$ [F23.1; U. D. Staerz *et al., J. Immunol.* **134**, 3994 (1985)]; $V_\beta11$ [RR3-15; J. Bill *et al., J. Exp. Med.* **169**, 1405 (1989)]; and $V_\beta13$ [MR12.4; D. M. Zaller *et al., J. Exp. Med.* **171**, 1943 (1990)] were used for staining of cells with appropriate fluorescein isothiocyanate (FITC)–labeled second antibodies. Some studies were performed with FITC- or biotin-labeled antibodies to $V_\beta3$ specificities $V_\beta5$, $V_\beta9$, and $V_\beta13$ (PharMingen, San Diego, CA). Analyses were performed on a FACS 440.
26. We gratefully acknowledge the excellent technical assistance of E. Rudikoff, the skillful FACS assistance provided by F. Hausman, and the help of Ms. N. Lofgren in the preparation of the manuscript. We thank R. J. Hodes, J. W. Hartley, B. J. Fowlkes, G. M. Shearer, and C. A. Janeway, Jr., for critical reviews of the manuscript and numerous helpful conversations during the course of these studies and S. K Ruscetti for contributions to the protein analyses. This work was supported in part by contract NO1-AI-72622 to Microbiological Associates, Inc., Bethesda, MD.

13 December 1990; accepted 20 February 1991

Engraftment and Development of Human T and B Cells in Mice After Bone Marrow Transplantation

IDO LUBIN, YIFAT FAKTOROWICH, TSVEE LAPIDOT, YEHUDIT GAN, ZELIG ESHHAR, EPHRAIM GAZIT, MIA LEVITE, YAIR REISNER*

A model for human lymphocyte ontogeny has been developed in a normal mouse. Human bone marrow, depleted of mature T and B lymphocytes, and bone marrow from mice with severe combined immunodeficiency were transplanted into lethally irradiated BALB/c mice. Human B and T cells were first detected 2 to 4 months after transplantation and persisted for at least 6 months. Most human thymocytes (30 to 50 percent of total thymocytes) were CD3$^+$CD4$^+$CD8$^+$. Human immunoglobulin was detected in some chimeras, and a human antibody response to dinitrophenol could be generated after primary and secondary immunization.

XENOGENEIC BONE MARROW (BM) transplants are possible between closely related species, such as rat and mouse (*1*). Human hematopoietic stem cells can also be transplanted into mice, without the occurrence of graft-versus-host disease, if the mice are genetically immune-deficient; examples of such mice being those with severe combined immune deficiency (SCID) (*2, 3*) and Bg/Nu/Xid mice (*4*). Although the engraftment of human cells in these models permits the infection of mice with human immunodeficiency virus (HIV) (*5*), the degree of chimerism after transplantation is limited and transient. Transplants of hematopoietic stem cells from human fetal liver into SCID mice do not engraft or differentiate into T cells unless the transplant has been supplemented with a graft of human fetal thymic epithelium implanted under the kidney capsule. Furthermore, human lymphocytes are not detected in significant numbers in the peripheral blood beyond 2 to 3 months (*2, 3*).

After total body irradiation, normal mice die within 2 weeks if a BM transplant is not administered. T cell differentiation in mice occurs within approximately 14 to 21 days after BM transplantation. In clinical studies of transplants of T cell–depleted BM into SCID (*6*) or leukemic patients (7), differentiation of human stem cells into mature T cells is slow, requiring from 1 to 4 months. Clearly, in a murine microenvironment, human stem cell differentiation may be inefficient and even slower because of the low cross-reactivity between murine and human cytokines and other molecules that are important in cellular recognition. The conflict between the need for prompt hematopoietic reconstitution and the slow differentiation rate of human T and B cells was resolved in the present study by the use of SCID mice as BM donors rather than as recipients. BM cells from SCID mice can promptly reconstitute all hematopoietic lineages except T and B cells (*8*). To test whether the "empty space" in the thymus and BM could gradually be occupied by, and populated with, normal human T and B cells, we combined a transplant of SCID mouse BM with a transplant of human BM that had been depleted of both T and B cells by differential agglutination with soybean agglutinin (SBA$^-$) and by subsequent removal of residual CD2$^+$ lymphocytes that form rosettes with sheep erythrocytes (E$^-$) (*9*). Because SCID in mice does not always result in an absolute deficiency of T cells, BM from the SCID mice was also depleted of T cells to maximize the likelihood that the lymphoid compartment in the chimeric mice would be derived from human lymphocytes and their progenitors.

Our approach allowed human T and B cells to develop in the peripheral blood of lethally irradiated, normal BALB/c mice that were of the same H-2 haplotype as the SCID BM donors. A total of 31 mice in four series of experiments were transplanted with BM from humans and SCID mice. Of the 31 mice transplanted, 20 survived for three or more months. Infection killed 10 of the 31 mice, mainly during the first 3 weeks after transplantation. Sixteen of the 20 survivors contained human T cells or human B cells, or both. Fourteen of the 20 surviving mice had both human T and B cells in significant numbers in the peripheral blood.

The first series of mice was initially evaluated for T cell engraftment with the use of cytofluorometry to measure the appearance of human CD3$^+$ T cells in the peripheral blood. One mouse (no. 3) showed the presence of human T cells 7 weeks after the transplant (Fig. 1A, inset); a monoclonal

I. Lubin, Y. Faktorowich, T. Lapidot, Y. Gan, M. Levite, Y. Reisner, Department of Biophysics, Weizmann Institute of Science, Rehovot 76100, Israel.
Z. Eshhar, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot 76100, Israel.
E. Gazit, Department of Pediatrics, Chaim Sheba Medical Center, Tel HaShomer 62621, Israel.

*To whom correspondence should be addressed.

antibody (MAb) to human CD3 bound 25% of the peripheral blood lymphocytes (PBLs) of this mouse, compared to 66% of normal human PBLs and 0.9% of PBLs from a mouse transplanted only with SCID mouse BM.

By week 10 after the transplant, low numbers of T cells were found in four of seven mice tested, ranging from 6.5 to 10.8% of PBLs (mean of 7.9%). T cell numbers increased, so that after 4 months all seven mice showed the presence of human $CD3^+$ T cells. Human T cells constituted more than 10% (mean of 27.1%, range of 11.1 to 54.1%) of PBLs in six of seven mice tested. These percentages were confirmed with MAbs to human CD2. Most human T cells (49 to 84%) were of abnormal or immature phenotypes, such as $CD3^+CD2^+CD4^-CD8^-$, 5 months after the transplant, similar to the situation with leukemia patients shortly after successful BM transplantation with T cell–depleted bone marrow (*6*). The remainder of the human T cells were $CD3^+CD4^+CD8^-$ and $CD3^+CD4^-CD8^+$. Thus, we achieved a slow but steady increase in human T cells, which began 7 weeks after transplantation and reached a maximal level after 4 to 5 months (Fig. 1A); a pattern that is similar to the slow rate of T cell differentiation in humans with SCID or leukemia after transplantation of SBA^-E^- BM (*6*).

A significant population of human lymphocytes, mostly of the $CD4^+CD8^+$ phenotype, was detected by cytofluorometry in the thymus 5 (Fig. 1, B and C) or 6 months after transplantation. Smaller populations of $CD4^+CD8^-$ and $CD4^-CD8^+$ human lymphocytes were also detected.

Five months after transplantation, splenocytes from human-mouse chimeras (series 3) were stimulated with OKT3, a MAb to CD3, under conditions that are used to test immune reconstitution in SCID or leukemia patients after BM transplantation. OKT3 selectively stimulates human T cells and does not cross-react with murine cells. After 7 days of culture, stimulation indexes were 3.1 and 2.2 for two chimeric mice tested, compared to 2.2 for human PBLs and 0.8 for normal mouse splenocytes. However, the magnitude of the response of human-mouse chimeric splenocytes (the difference between the averages of thymidine incorporation obtained in the presence and in the absence of OKT3) was only 10% (3178 cpm) and 7% (2440 cpm), respectively, of the normal human PBL response (32,707 cpm). When splenocytes from the same chimeras were incubated with interleukin-2 (IL-2)–containing medium and assayed after 7 days of culture for $CD3^+$ human T cells, most cells (55%) were $CD3^+$ (Fig. 2); when normal human PBLs were cultured under these conditions, 83% were $CD3^+$, whereas 1% of splenocytes from a control mouse transplanted with only SCID BM were $CD3^+$.

Human B cells in the peripheral blood of

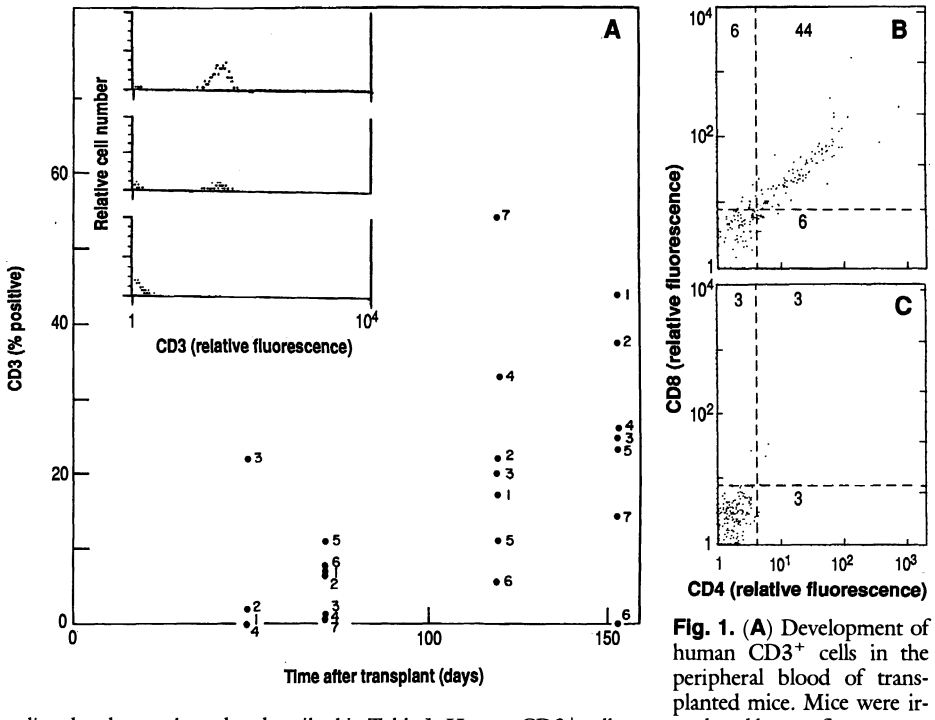

Fig. 1. (**A**) Development of human $CD3^+$ cells in the peripheral blood of transplanted mice. Mice were irradiated and transplanted as described in Table 1. Human $CD3^+$ cells were analyzed by cytofluorometry with an antibody to CD3 (Leu4). Numbers 1 to 7 refer to surviving mice of series 1 (Table 1). (Inset) A typical experiment performed on day 49 after transplantation. Staining of normal human PBLs with Leu4 (upper section) is compared to staining of PBLs from mouse no. 3 (middle section) and from a control mouse transplanted with SCID BM alone (lower section). Staining of normal mouse PBLs was identical to that of the control mouse. (**B**) Double staining of thymocytes from a mouse transplanted with human BM, with MAbs to human CD4 and human CD8. The thymuses of mouse no. 7 and no. 1 from series 1 were removed 5 and 6 months after the transplant, respectively, and the thymocytes were washed twice in phosphate-buffered saline (approximately 1×10^6 to 2×10^6 viable cells could be collected after washing) and simultaneously stained with directly labeled, phycoerythrine-conjugated MAb T4 and fluorescein isothiocyanate (FITC)–conjugated T8. The cells were then washed and analyzed by cytofluorometry on a FACS-Scan (Becton-Dickinson, Mountain View, California). (**C**) A control of normal BALB/c murine thymocytes and the same MAbs as in (B). Percentages of single or double positives without subtraction of background staining are denoted in (B) and (C). The percentages of human $CD4^+CD8^+$, $CD4^+CD8^-$, and $CD4^-CD8^+$ cells (after subtraction of background staining from the control experiment) in the thymus of mouse no. 7 were 41.3, 3.7, and 3.1%, respectively. In the thymus of mouse no. 1, these percentages were 42, 11.7, and 8.2%, respectively. The percentages of human $CD2^+$ cells in the thymuses of mice nos. 1 and 7 were 35.0 and 47.1%, respectively; the percentages of human $CD3^+$ cells were 32.0 and 43.0%, respectively.

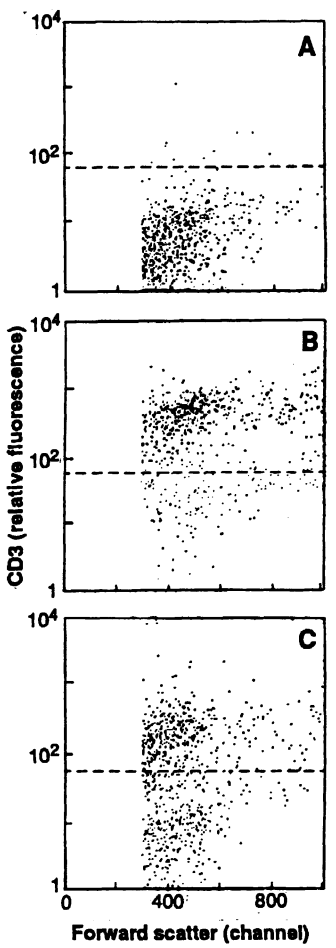

Fig. 2. $CD3^+$ splenocytes after 7 days of culture with IL-2. (**A**) Spleen cells from a control mouse transplanted with SCID BM alone. (**B**) Normal human PBLs. (**C**) Spleen cells of a human-mouse chimera (no. 2 of series 1, 9 months after BM transplantation). Cells (25×10^3 cells per well) were cultured for 7 days in RPMI medium containing fetal calf serum (10%) and IL-2 (20 U per well).

transplanted mice were detected with directly labeled MAbs to the human B cell differentiation antigens CD19 and CD20. This test was performed in mice from series 1, 5 and 6 months after transplantation. Human $CD19^+$ and $CD20^+$ cells ranged from 4.5 to 16.3% of PBLs for CD19 and from 3.5 to 33.0% for CD20 after 5 months (Fig. 3, A to E), and from 9.0 to 30.4% and from 2.1 to 20.6%, respectively, after 6 months. Human B cells were first detected 9 weeks after transplantation in four out of seven mice tested from series 3 (Table 1); $CD19^+$ cells constituted 2.4 to 14.3% of PBLs in these mice.

The function of the human B cells was assessed both by measuring human immunoglobulin (Ig) in serum and by titrating human antibodies generated in response to dinitrophenol conjugated to keyhole limpet hemocyanin (DNP-KLH). Among mice transplanted with human BM depleted of both T and B cells, seven of 20 engrafted mice were positive for human Ig. Of the positive animals, human IgM was detected in two of four mice and human IgG in the other two mice. In subsequent experiments, mice were also transplanted with unmodified BM (Table 1 and below); 8 of 22 of these mice were positive for human Ig; human IgM was detected in two mice and human IgG in the other six animals.

We immunized four chimeric mice that were positive for human Ig (two mice from series 1 and two mice from series 3, 8 and 6 months after transplantation, respectively) with DNP-KLH; both primary and secondary responses specific for DNP were induced in two of four animals (Fig. 3F). After secondary immunization, specific human IgG and IgM could be detected in one of two mice. These results suggest that engrafted human B cells may be able to function in a murine environment. It remains to be determined, however, whether the human B cells that responded to DNP-KLH were derived from residual small populations of mature B cells or from progenitors in the SBA^-E^- BM that developed within the murine microenvironment. The nature and species of antigen-presenting cells in these chimeras are also unclear. Investigation of the restrictive patterns and growth requirements of functional T and B cells may suggest modifications of the model that will increase the proportion of animals achieving chimerism and enhance the levels of function observed.

Murine PBLs did not cross-react with the MAbs to human cells used in our study. We also confirmed the presence of human lymphocytes in the peripheral blood of transplanted mice by DNA analysis (Fig. 4). Genomic DNA of the human lymphocyte antigen DQβ genes was detected in the peripheral blood of a transplanted mouse, whereas normal PBLs from an untreated mouse were negative. In the same sample of PBLs from the transplanted mouse (taken 6 months after transplantation), MAbs to CD3 and CD19 revealed that 60% of the PBLs were human T and B cells, whereas a MAb to murine $H\text{-}2^d$ detected only 6.2% of the cells.

Because of the presence of normal myeloid and erythroid precursors in the BM of SCID mice, we hypothesized that when human SBA^-E^- BM cells were transplanted together with murine SCID BM cells, engraftment of human myeloid cells would be negligible. However, although BM prepara-

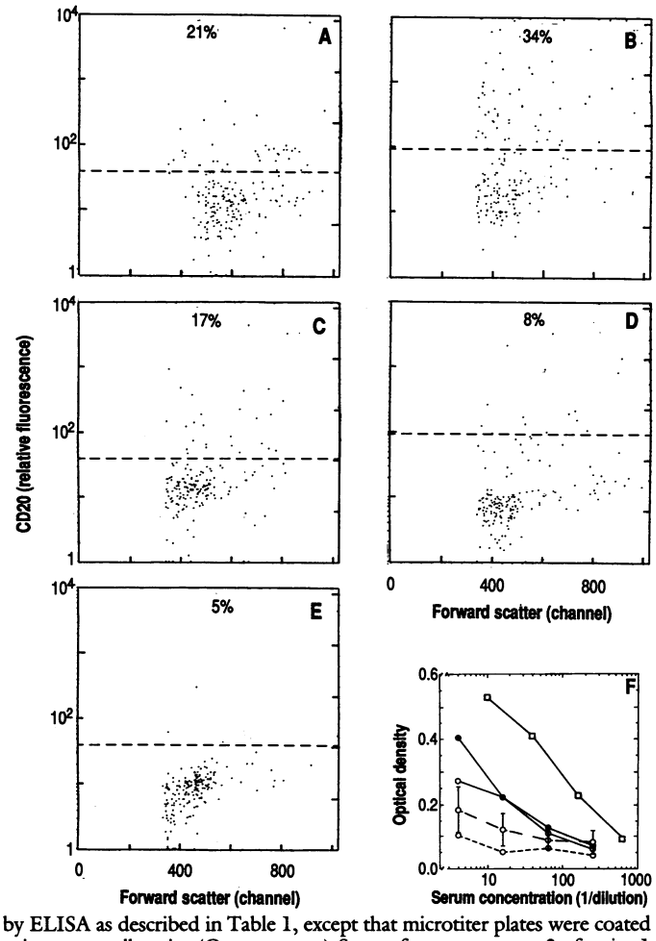

Fig. 3. PBLs (**A**) and splenocytes (**B**) of mouse no. 7 of series 1 were examined 5 months after transplantation with FITC-conjugated MAb to human CD20 and analyzed as described in Fig. 1. Results were compared to those for normal human PBLs (**C**), normal mouse splenocytes (**D**), and normal mouse PBLs (**E**). Percentage of positive cells (above marked threshold) are shown at the top. (**F**) DNP-specific human Ig in human-mouse chimeras immunized with DNP-KLH. Human-mouse chimeras whose PBLs were positive for both human T and B cells, and control mice transplanted with SCID BM alone, were injected subcutaneously with 20 μg of DNP-KLH emulsified in complete Freund's adjuvant. After 48 days, some of the primed and control mice were injected subcutaneously with 100 μg of DNP-KLH emulsified in complete Freund's adjuvant. Blood was removed from mice 6 days later and antibodies to DNP were measured by ELISA as described in Table 1, except that microtiter plates were coated with DNP conjugated to bovine serum albumin. (Open squares) Serum from mouse no. 2 of series 1 (challenged twice); (open circles, solid line) serum from mouse no. 3 of series 1 (challenged twice); (solid circles) serum of a mouse from series 3 (challenged once); (open circles, short dashes) serum of a control mouse (challenged twice); (open circles, long dashes, error bars) mean (± SD) of 15 sera collected from control mice (transplanted with SCID BM alone and challenged once with DNP-KLH).

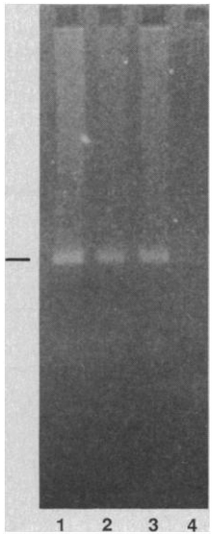

Fig. 4. DNA analysis of peripheral blood from a human-mouse chimera 6 months after BM transplantation. PBLs were lysed with distilled water and boiled for 10 min. Debris was sedimented by centrifugation, and the genomic DNA in the supernatant was amplified by the polymerase chain reaction (PCR) procedure for 30 cycles, with *Taq* DNA polymerase (Perkin-Elmer Cetus, Emeryville, California) (*16*). The reaction mixture, which included 500 ng of genomic DNA, 20 pmol of the DQβ primers GLPDQβ1 and GAMPDQβ2 (*17*), and 2 U of *Taq* DNA polymerase, was subjected to repeated cycles of denaturation of 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s. The products were analyzed by 2% agarose gel electrophoresis, and the amplification was regarded as successful only if the control sample from human PBLs showed a unique 246–base pair band (*18*). Lane 1, normal human PBLs; lane 2, an artificial mixture of human and mouse PBLs (ratio 1:3); lane 3, PBLs from a mouse transplanted with human BM (mouse of series 1); lane 4, PBLs from an untreated BALB/c mouse.

tions obtained from human-mouse chimeras at various times after transplantation have been predominantly of mouse origin when analyzed with a MAb to H-2^d (specific for SCID and BALB/c mice), we repeatedly found cells (1 to 10%) positive for human myeloid differentiation antigens (CD13 and CD33) in PBLs; such cells were also detected in samples of BM and spleen. Whether the cells detected include clonogenic progenitors with significant capacity for self-renewal and differentiation was not determined.

We also assessed whether it was necessary to deplete the human BM of T cells for long-term engraftment of human T and B cells to occur. With the same transplantation approach, but with the transfer of more unmodified BM cells (8×10^7 cells per mouse) (to provide a comparable number of progenitor cells), we observed a rate of engraftment similar to that achieved with T cell–depleted bone marrow (Table 1) and without manifestations of graft-versus-host disease. In contrast, in a separate group of four animals that were transplanted with an intravenous infusion of 4×10^7 peripheral blood mononuclear cells (PBMs) rather than BM cells, no animals developed detectable lymphoid chimerism during a 4-month period after transplantation.

The disparity in the incidence of durable chimerism after transplants of T cell–depleted BM when compared with transplants of unmodified PBMs, the demonstration that most human lymphocytes in the thymuses of transplanted mice were $CD3^+CD4^+CD8^+$, and the late emergence of human $CD3^+CD4^+CD8^-$ and $CD3^+CD4^-CD8^+$ cells in the peripheral blood of engrafted mice suggest the possibility that the human lymphocytes detected in the blood developed within the murine thymic microenvironment from less mature lymphoid populations. However, it is still possible that a residual subpopulation of mature T cells in the T cell–depleted BM propagated extrathymically.

The reason for the difference between our results and the lack of development of detectable human T cells after transplants of human fetal liver into SCID mice in the absence of human thymic epithelium (*2*) is unclear, but may be explained in part by quantitative differences in the dosage of hematopoietic progenitor cells provided, because stem cell inoculum size is a factor in the engraftment of both fetal liver and T cell–depleted BM allografts (*1, 10*). SCID mice also retain populations of cells, such as natural killer cells, that may contribute to BM rejection (*11, 12,*) during the initial period after transplantation. This form of graft resistance has been reported in murine and human forms of SCID (*12, 13*). Natural killer cell–mediated graft resistance can be overcome by high doses of cells in the transplant inoculum and may also be less pronounced in the lethally irradiated normal BALB/c recipient mice used in our study.

The protection from hematopoietic death provided by the transplant of murine SCID BM allows for the slow generation of human T and B cells in transplanted mice. This approach could probably be extended and broadened with the use of other genetically deficient murine BM donors or recipients that may allow for the engraftment and emergence of different human cell populations in lethally irradiated normal mice.

Table 1. Engraftment of human T and B cells into lethally irradiated BALB/c mice. Eight- to 12-week-old female BALB/c mice (Olac Farms, Bicester, United Kingdom) were exposed to a single dose of 10 Gy of total body irradiation from a Gammabeam 150-A ^{60}Co source (Atomic Energy of Canada, Kanata, Ontario) with a focal skin distance of 75 cm at a rate of 0.7 Gy/min. One day later, 10^7 T cell–depleted human BM cells were intravenously transferred into each mouse. After a further 24 hours, T cell–depleted BM cells were prepared from 8- to 12-week-old male SCID mice (Weizmann Institute Animal Breeding Center, Rehovot, Israel) (*14, 15*), and 2×10^6 cells were transplanted into the same recipients. Blood was removed from mice from the retro-orbital vein with the use of heparin-coated glass capillaries, and mononuclear cells were purified by Ficoll-Hypaque (Pharmacia) fractionation. Human T cells were detected by directly labeled MAbs, including T3 (to CD3), T4 (to CD4), T8 (to CD8), and T11 (to CD2) (Coulter Immunology, Luton, United Kingdom) and Leu4 (to CD3) (Becton-Dickinson). Human B cells were detected by directly labeled MAbs B1 (to CD20) and B4 (to CD19) (Coulter Immunology). These MAbs did not cross-react with normal mouse PBLs. Mice were scored as positive when more than 2% of their PBLs were stained by the appropriate MAb (after subtraction of background staining of PBLs from normal BALB/c mice). The 2% threshold was established on the basis of experiments in which the MAbs were tested on PBLs from normal BALB/c mice, BALB/c mice transplanted with 2×10^6 T cell–depleted BM cells from SCID donors, and humans; after subtraction of background staining found on cells from the first group, the use of a 2% cutoff effectively avoided "false positives" in the second group. N.D., not determined. Total human immunoglobulin (Ig) in the serum of transplanted mice was measured 3 to 8 months after transplantation with a double-antibody enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with affinity-purified goat Fab fragments of antibodies to human Ig (50 μg/ml) (BioMakor, Rehovot, Israel), nonspecific binding of protein was eliminated by incubation with a 1% solution of bovine serum albumin, and diluted (1:1) serum from transplanted mice was then added. The wells were washed and then incubated with peroxidase-conjugated goat antibodies to human Ig (IgG, IgM, IgA) (Zymed Laboratory, South San Francisco, California) after the antibodies were incubated with normal mouse serum (1:1) (to avoid cross-reactivity with mouse Ig). After further washes, substrate solution [2,2′-azinodi-(3-ethylbenzthiazoline sulfonic acid) diammonium salt (ATBS) (Sigma)] was added and the optical density at 620 nm was determined by a Titerteck Multiscan-ML (Flow Labs.) ELISA reader. Readings corresponding to values greater than 20 ng/ml were recorded as positive (ranging from 20 ng/ml to 3.0 μg/ml), on the basis of control experiments in which serum of mice transplanted with SCID BM alone were negative. Human IgG and IgM were determined in some mice as described above with the use of peroxidase-conjugated horse antibodies to human IgG Fc fragments (BioMakor) and peroxidase-conjugated goat antibodies to human IgM(μ) (BioMakor).

Time after transplant (weeks)	Mice surviving	Mice with human PBLs			Mice with human Ig
		T cells	B cells	T + B cells	
			Series 1		
7	7/10	1/7	N.D.	N.D.	N.D.
10	7/10	4/7	N.D.	N.D.	N.D.
16	7/10	7/7	N.D.	N.D.	N.D.
22	7/10	6/6	6/6	6/6	3/6
			Series 2		
7	3/6	0/3	N.D.	N.D.	N.D.
16	3/6	2/3	1/3	1/3	0/3
			Series 3		
9	7/8	4/7	4/7	4/7	3/7
			Series 4		
11	4/7	3/4	4/4	3/4	1/4
			*Series 5**		
11	7/8	6/7	5/7	5/7	2/7
			Series 6†		
11	8/14	3/8	7/8	2/7	5/8
			Series 7‡		
11	7/7	5/7	7/7	5/7	1/7

*Intravenous injection of unseparated human BM cells (8.0×10^7 cells per mouse). †Intraperitoneal injection of unseparated human BM cells (2.0×10^7 cells per mouse). ‡Intravenous injection of mononuclear human BM cells (1.5×10^7 cells per mouse).

REFERENCES AND NOTES

1. D. W. van Bekkum and B. Lowenberg, *Bone Marrow Transplantation: Biological Mechanisms and Clinical Practice* (Dekker, New York, 1985), pp. 311–350.
2. J. M. McCune *et al.*, *Science* **241**, 1632 (1988).
3. R. Namikawa, K. N. Weilbaecher, H. Kaneshima, E. J. Yee, J. M. McCune, *J. Exp. Med.* **172**, 1055 (1990).
4. S. Kamel-Reid and J. E. Dick, *Science* **242**, 1706 (1988).
5. R. Namikawa, H. Kaneshima, M. Lieberman, I. L. Weissman, J. M. McCune, *ibid.*, p. 1684.
6. Y. Reisner *et al.*, *Blood* **61**, 341 (1983).
7. C. A. Keever *et al.*, *ibid.* **73**, 1340 (1989).
8. W. Schuler *et al.*, *Cell* **46**, 963 (1986).
9. Y. Reisner *et al.*, *Lancet* **ii**, 327 (1981).
10. T. Lapidot, A. Terenzi, T. S. Singer, O. Salomon, Y. Reisner, *Blood* **73**, 2025 (1989).
11. R. Kiessling *et al.*, *Eur. J. Immunol.* **7**, 655 (1977).
12. W. J. Murphy, V. Kumar, M. Bennett, *J. Exp. Med.* **165**, 1212 (1987).
13. R. J. O'Reilly *et al.*, in *Preliminary Immunodeficiency Diseases*, M. M. Eibl and F. S. Rosen, Eds. (Elsevier, New York, 1986), pp. 301–307.
14. Y. Reisner, L. Itzicovitch, A. Meshorer, N. Sharon, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2933 (1978).
15. E. Schwartz, T. Lapidot, D. Gozes, T. S. Singer, Y. Reisner, *J. Immunol.* **138**, 460 (1987).
16. R. K. Saiki *et al.*, *Science* **239**, 487 (1988).
17. J. A. Todd, J. I. Bell, H. O. McDevitt, *Nature* **329**, 599 (1987).
18. K. S. Ronningen, T. Iwe, T. S. Halstensen, A. Spurkland, E. Thorsby, *Hum. Immunol.* **26**, 215 (1989).
19. We thank M. Martelli, F. Aversa, A. Terenzi, and W. Friedrich for the samples of human BM; B. Morgenstern, G. Black, and D. Ochert for editorial assistance; and R. O'Reilly for critical review of the manuscript. Supported in part by a grant from the Yeda Fund of the H. Levine Center for Applied Research, the Weizmann Institute of Science.

19 November 1990; accepted 7 February 1991

Adenovirus-Mediated Transfer of a Recombinant α1-Antitrypsin Gene to the Lung Epithelium in Vivo

MELISSA A. ROSENFELD, WOLFGANG SIEGFRIED, KUNIHIKO YOSHIMURA, KOICHI YONEYAMA, MASASHI FUKAYAMA, LARUE E. STIER, PAAVO K. PÄÄKKÖ, PASCALE GILARDI, LESLIE D. STRATFORD-PERRICAUDET, MICHEL PERRICAUDET, SOPHIE JALLAT, ANDREA PAVIRANI, JEAN-PIERRE LECOCQ, RONALD G. CRYSTAL*

The respiratory epithelium is a potential site for somatic gene therapy for the common hereditary disorders α1-antitrypsin (α1AT) deficiency and cystic fibrosis. A replication-deficient adenoviral vector (Ad-α1AT) containing an adenovirus major late promoter and a recombinant human α1AT gene was used to infect epithelial cells of the cotton rat respiratory tract in vitro and in vivo. Freshly isolated tracheobronchial epithelial cells infected with Ad-α1AT contained human α1AT messenger RNA transcripts and synthesized and secreted human α1AT. After in vivo intratracheal administration of Ad-α1AT to these rats, human α1AT messenger RNA was observed in the respiratory epithelium, human α1AT was synthesized and secreted by lung tissue, and human α1AT was detected in the epithelial lining fluid for at least 1 week.

ONE OF THE HURDLES TO OVERcome in most forms of somatic gene therapy is the specific delivery of the therapeutic gene to the organs manifesting the disease. The lung presents special advantages because a functional gene can be delivered directly to the respiratory epithelium by means of tracheal instillation. The disadvantage of such an approach is due to the normal biology of the respiratory epithelium; only a small proportion of alveolar and airway epithelial cells go through the proliferative cycle in 1 day, and a large proportion of the cells are terminally differentiated and are, therefore, incapable of proliferation (*1*). In this regard, it may be difficult to transfer functional genes to the respiratory epithelium by means of vectors (such as retroviruses) that require proliferation of the target cells for expression of the newly transferred gene (*2*).

To circumvent the slow target-cell proliferation, we have used a recombinant adenoviral vector to transfer a recombinant human gene to the respiratory epithelium in vivo. Host cell proliferation is not required for expression of adenoviral proteins (*3, 4*), and adenoviruses are normally trophic for the respiratory epithelium (*5*). Other advantages of adenoviruses as potential vectors for human gene therapy are as follows: (i) recombination is rare; (ii) there are no known associations of human malignancies with adenoviral infections despite common human infection with adenoviruses; (iii) the adenovirus genome (which is a linear, double-stranded piece of DNA) can be manipulated to accommodate foreign genes of up to 7.0 to 7.5 kb in length; and (iv) live adenovirus has been safely used as a human vaccine (*3–8*).

The adenovirus (Ad) major late promoter (MLP) was linked to a recombinant human α1AT gene (*9*) and was incorporated into a replication-deficient recombinant (Fig. 1) (*5, 10*). The vector has a deletion of a portion of the E3 region (that permits encapsidation of the recombinant genome containing the exogenous gene) and a portion of the viral E1a coding sequence (that impairs viral replication) but contains an insert of an α1AT expression cassette (Fig. 1) (*10, 11*). After packaging into an infectious, but replication-deficient virus, Ad-α1AT is capable of directing the synthesis of human α1AT in Chinese hamstery ovary (CHO) and human cervical carcinoma (HeLa) cell lines (*10*).

We obtained tracheobronchial epithelial cells by brushing the epithelial surface of the tracheobronchial tree from the lungs of the cotton rat [*Sigmodon hispidus*, an experimental animal used to evaluate the pathogenesis of respiratory tract infections caused by human adenoviruses (*12*)]. The freshly removed cells infected in vitro with Ad-α1AT expressed human α1AT mRNA transcripts, as demonstrated by in situ hybridization with a ^{35}S-labeled antisense human α1AT RNA probe (Fig. 2). In contrast, no human α1AT mRNA transcripts were observed in uninfected, freshly isolated tracheobronchial epithelial cells. Human α1AT mRNA transcripts in the infected cells were capable of directing the synthesis and secretion of human α1AT, as shown by biosynthetic labeling and immunoprecipitation with a specific antibody to human α1AT (Fig. 2E). The newly synthesized, secreted α1AT was human α1AT, as shown by the fact that human α1AT (Fig. 2E, lane 3), but not cotton rat serum, blocked the antibody to human α1AT.

Ad-α1AT transferred the recombinant α1AT gene to the cotton rat lung in vivo (Fig. 3). Human α1AT transcripts were observed in the lungs 2 days after intratracheal instillation of Ad-α1AT, but not in lungs of animals that received only phosphate-buffered saline (PBS) or in lungs of animals that received the Ad5 E1a-deletion mutant, Ad-dl312 (*13*). Biosynthetic labeling and immunoprecipitation of extracellular protein from lung fragments removed from infected animals demonstrated that de novo synthesis and secretion of human α1AT also occurred (Fig. 3B, lanes 11

M. A. Rosenfeld, W. Siegfried, K. Yoshimura, K. Yoneyama, M. Fukayama, L. E. Stier, P. K. Pääkkö, R. G. Crystal, Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892.
P. Gilardi, L. D. Stratford-Perricaudet, M. Perricaudet, Institut Gustave Roussy, Centre National de la Recherche Scientifique Unité Associée 1301, 94805 Villejuif Cedex, France.
S. Jallat, A. Pavirani, J.-P. Lecocq, Transgene SA, 67082 Strasbourg, France.

*To whom correspondence should be addressed.