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SUSTAINED CORRECTION OF X-LINKED SEVERE COMBINED IMMUNODEFICIENCY BY EX VIVO GENE THERAPY

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ABSTRACT

Background X-linked severe combined immunodeficiency due to a mutation in the gene encoding the common γ (γ c) chain is a lethal condition that can be cured by allogeneic stem-cell transplantation. We investigated whether infusion of autologous hematopoietic stem cells that had been transduced in vitro with the γ c gene can restore the immune system in patients with severe combined immunodeficiency.

Methods CD34+ bone marrow cells from five boys with X-linked severe combined immunodeficiency were transduced ex vivo with the use of a defective retroviral vector. Integration and expression of the γ c transgene and development of lymphocyte subgroups and their functions were sequentially analyzed over a period of up to 2.5 years after gene transfer.

Results No adverse effects resulted from the procedure. Transduced T cells and natural killer cells appeared in the blood of four of the five patients within four months. The numbers and phenotypes of T cells, the repertoire of T-cell receptors, and the in vitro proliferative responses of T cells to several antigens after immunization were nearly normal up to two years after treatment. Thymopoiesis was documented by the presence of naive T cells and T-cell antigen-receptor episomes and the development of a normal-sized thymus gland. The frequency of transduced B cells was low, but serum immunoglobulin levels and antibody production after immunization were sufficient to avoid the need for intravenous immunoglobulin. Correction of the immunodeficiency eradicated established infections and allowed patients to have a normal life.

Conclusions Ex vivo gene therapy with γ c can safely correct the immune deficiency of patients with X-linked severe combined immunodeficiency. (N Engl J Med 2002;346:1185-93.)

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DEFICIENCY of the common γ (γ c) chain, an X-linked disorder, causes the most frequent form of severe combined immunodeficiency disease.^{1,2} The γ c chain is an essential component of five cytokine receptors, all of which are necessary for the development of T cells and natural killer cells. Without the γ c chain, there is a complete absence of mature T and natural killer cells, whereas B cells are usually present in normal or increased numbers. Severe combined immunodeficiency is fatal during the first year of life because of severe, recurrent infections, unless transplantation of hematopoietic stem cells restores T-cell function.^{3,4} The survival rate after transplantation of HLA-identical hematopoietic stem cells is more than 90 percent, whereas with haploidentical stem cells it is 70 to 78 percent.^{3,4} In most patients, deficient B-cell function persists after transplantation and requires lifelong immune-globulin-replacement therapy.^{3,5} Some patients also have persistent deficiencies of T-cell function after stem-cell transplantation.^{4,6} Assessment of an al-

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ternative therapy based on the ex vivo transfer of the γ_c gene into autologous hematopoietic precursor cells was therefore warranted. In a preliminary report, we showed that this approach corrected the T-cell deficiency in two patients with X-linked severe combined immunodeficiency who were followed for 10 months after gene transfer.⁷ We now report the effectiveness of the procedure in five patients with a follow-up of up to 30 months.

METHODS

Patients

Five consecutive patients without HLA-identical donors were enrolled in the trial between March 1999 and February 2000. The main characteristics of these boys at the time of diagnosis are shown in Table 1. The diagnosis of X-linked severe combined immunodeficiency was based on peripheral-blood lymphocyte counts and confirmed by γ_c mutation analysis. The protocol was approved by the French Drug Agency and the local ethics committee, and written informed consent was obtained from the parents, who were told that an alternative treatment (bone marrow transplantation) was available. All of the patients were kept in sterile isolation and received nonabsorbable antibiotics and intravenous immune globulin. Additional information about the five patients is available as Supplementary Appendix 1 with the full text of this article at <http://www.nejm.org>.

Retrovirus-Mediated Transduction

The vector containing the γ_c chain was derived from a defective Moloney murine leukemia virus and has been previously described.⁷ With the patients under general anesthesia, 30 to 150

ml of bone marrow was obtained, and CD34+ cells in the marrow were selected for, as described below. These cells were stimulated to grow in X-vivo 10 medium (BioWhittaker, Walkersville, Md.) containing 4 percent fetal-calf serum (StemCell Technologies, Vancouver, B.C., Canada), 300 ng of stem-cell factor per milliliter (Amgen, Thousand Oaks, Calif.), 300 ng of Flt-3 ligand per milliliter (Immunex, Seattle), 60 ng of interleukin-3 per milliliter (Novartis, Rueil-Malmaison, France), and 100 ng of polyethylene glycol-conjugated megakaryocyte growth and differentiation factor per milliliter (Amgen). The cells were then transduced with a supernatant of the cultured γ_c -containing vector in the presence of the preceding cytokines and 4 ng of protamine sulfate per milliliter (Choay Sanofi, Gentilly, France). The procedure was carried out in sterile bags (Nexell Therapeutics, Irvine, Calif.) that were coated with 50 ng of human recombinant fibronectin per milliliter (Takara Shuzo, Shiga, Japan). The supernatant was replaced every 24 hours during the three-day transduction period. The number of cultured cells was increased by a factor of five to eight, and 14 million to 38 million CD34+ cells per kilogram of body weight were infused into the patients without preparative conditioning (Table 1).

Analysis of Immune Reconstitution

Immunofluorescence analysis, assays for proliferation of peripheral-blood mononuclear cells, analysis of the T-cell-receptor repertoire, and studies of natural-killer-cell cytotoxicity were performed as previously described.^{7,9} The presence of serum antibodies against polioviruses, tetanus and diphtheria toxoids, *Haemophilus influenzae*, and *Streptococcus pneumoniae* was determined by enzyme-linked immunosorbent assays. Levels of isohemagglutinins were measured by a hemagglutination assay. Antibody levels were determined one to three months after three immunizations had been administered. The interval between the last intravenous infusion of immune globulin and the determination of antibody levels was at least three months.

TABLE 1. CHARACTERISTICS OF THE PATIENTS.

PATIENT No.	AGE AT TREATMENT	CLINICAL STATUS BEFORE TREATMENT	ENGRAFTMENT OF MATERNAL T CELLS	MUTATION	γ_c EXPRESSION BEFORE TREATMENT	INFUSED CELLS		CLINICAL STATUS AFTER TREATMENT	FOLLOW-UP
						CD34+	CD34 γ_c +		
	mo		cells/mm ³			cells/kg			yr
1	11	<i>Pneumocystis carinii</i> pneumonitis Protracted diarrhea Failure to thrive	0	Arg 289→stop	Yes	15 million	7 million–14 million	Well Normal growth	2.5
2	8	<i>Pneumocystis carinii</i> pneumonitis Protracted diarrhea Graft-versus-host disease–like lesions Failure to thrive	<10	Deletion of exon 6	No	16 million	5 million	Well Normal growth	2.3
3	10	Disseminated bacille Calmette–Guérin infection Adenovirus and respiratory syncytial virus infections in the lungs Protracted diarrhea Failure to thrive	0	Deletion of exon 4	No	14 million	5 million	Improving*	0.7
4	1	Well Free of infection	0	Tyr 219→stop	No	27 million	14 million	Well Normal growth	1.8
5	3	Graft-versus-host disease–like lesions	2000	Gln 285→Ala	No	38 million	20 million	Well Normal growth	1.6

*Eight months after gene therapy, Patient 3 underwent allogeneic stem-cell transplantation.

Leukocyte Subgroups and Purification of CD34+ Cells

Peripheral-blood samples were separated into mononuclear cells and granulocytes by centrifugation and sorted by flow cytometry (FACS Vantage, Becton Dickinson Immunocytometry Systems, San Jose, Calif.). Isolation of CD34+ progenitor cells was performed by an immunomagnetic procedure (Miltenyi Biotec, Bergisch Gladbach, Germany). Two successive immunomagnetic procedures increased the purity of the CD34+ population to 99 percent.

Quantification of Transgene Integration

Genomic DNA was extracted from peripheral-blood mononuclear cells and amplified with use of quantitative polymerase chain reaction (PCR). Amplification, data acquisition, and analysis were performed with the use of a sequence detector (ABI PRISM 7700, Perkin Elmer, Norwalk, Conn.). Two sets of primers and probes were used in each PCR reaction. For the quantification of integrated transgene sequences, the primers positioned in the long terminal repeat and probe were as previously described.¹⁰ The standard curve used as a reference for quantification of the viral copy number was based on serial dilutions of a plasmid ranging from 40 to 4 million copies. This plasmid contained two copies of the long terminal repeat and one of the human albumin sequence (Genethon III Laboratory, Evry, France).

To define the detection limit and linear range of duplex PCR, we used a standard curve consisting of a log-scale dilution of cells from an Epstein-Barr virus (EBV)-transformed B-cell line derived from a patient with X-linked severe combined immunodeficiency and containing approximately two copies of γ c provirus per cell with uninfected cells from the same EBV-transformed B cell line. The lower limit of sensitivity of the method was 0.01 percent of γ c-positive cells.

Quantification of T-Cell Antigen-Receptor Episomes

Analysis of T-cell antigen-receptor episomes in peripheral-blood mononuclear cells was performed by real-time quantitative PCR by means of the 5' nuclease assay (TaqMan) with an ABI PRISM 7700 system (Perkin Elmer).^{11,12} PCR conditions as well as primers and probe sequences are available on request.

Presence of Integrated Provirus after Long-Term Culture of CD34+ Cells

Purified CD34+ cells were cultured for six weeks on irradiated MS-5 stromal feeder layers in a limiting-dilution assay (10,000 to 150 cells per well) as described previously.¹³ After six weeks, the cells were assayed for colony-forming units. Subsequently, for each dilution, all colony-forming units obtained on day 14 from the same dish were pooled. DNA was analyzed by PCR to determine the percentage of γ c-positive dishes.

RESULTS

Clinical Outcome

After infusion of CD34+ cells that had been transduced in vitro with the γ c gene, four of the five patients (Patients 1, 2, 4, and 5) had a clear-cut clinical improvement (Table 1). Pulmonary infections in Patient 1 and Patient 2 cleared and did not recur, and graft-versus-host-like skin lesions, a feature of severe combined immunodeficiency, disappeared in Patient 2 and Patient 5 within the first 50 days after gene therapy. Patient 1 and Patient 2 left the sterile environment on day 90, and Patient 4 and Patient 5 left on day 45. In Patient 1 and Patient 2, protracted diarrhea resolved, and parenteral nutrition was discontinued four months and three months after gene

therapy, respectively. None of these four patients have subsequently had severe infections. Intravenous immune globulin was discontinued three to four months after gene therapy. Growth and psychomotor development have been normal to date. Patients 1, 2, 4, and 5 are now living at home in normal environmental conditions.

Patient 3, in whom reconstitution of T cells failed, underwent splenectomy four months after gene therapy for persistent splenomegaly caused by a disseminated bacille Calmette-Guérin infection. A rescue stem-cell transplantation from an unrelated donor matched at HLA-A, B, DR, and DQ loci but mismatched at one HLA-C locus was performed after eight months, according to the protocol. At the last follow-up visit, partial T-cell immunity had been restored in this patient.

T-Cell Development

In Patients 1, 2, and 4, the number of T cells increased progressively and reached normal values for age three to four months after gene therapy; they were within the normal range at the last follow-up visit (Fig. 1). In Patient 5, the initially high number of maternal T cells (Table 1) disappeared within three months after treatment, while autologous T cells appeared.

Quantitative analysis of provirus integration indicated that 100 percent of the T cells from Patients 1, 2, 4, and 5 contained the transgene (Fig. 2). On Southern blotting, there were one to three provirus integration sites per cell (data not shown). All T cells in Patients 2, 4, and 5 expressed cell-surface recep-

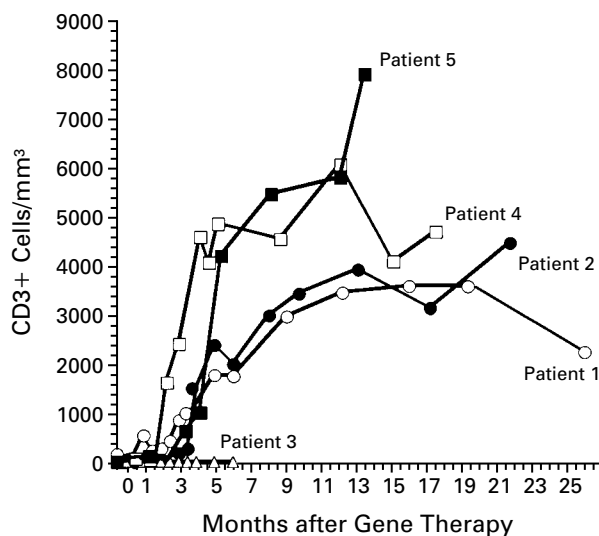


Figure 1. Absolute Numbers of CD3+ Cells after Gene Transfer in Patients 1 through 5.

tors with the γ c chain. In all four patients, there was a normal distribution of T cells with α/β or γ/δ receptors, and the numbers of CD4+ and CD8+ T cells were similar to those in age-matched controls (data not shown). Conversely, no T cells were detected in the blood of Patient 3 up to six months after treatment (Fig. 1).

Analysis of naive (CD45RA+) and memory (CD45RO+) subgroups within CD4+ and CD8+ populations showed that most T cells had the phenotype of naive CD45RA+ T cells (Fig. 3A). We also assessed whether T cells were being synthesized by measuring the level of T-cell antigen-receptor episomes. Intrathymic rearrangements of genes encoding T-cell antigen receptors cause the formation of extrachromosomal DNA episomes, which mark T cells that have recently emigrated from the thymus to the

periphery. As shown in Figure 3B, T-cell antigen-receptor episomes in Patients 1, 2, and 4 were first detected between day 60 and day 90, reached values found in age-matched controls, and remained stable for up to two years after gene transfer. Thirteen months after treatment, Patient 5 had 5500 CD45RA+ CD4+ T cells per cubic millimeter and 21,000 T-cell antigen-receptor episomes per 100,000 peripheral-blood mononuclear cells, respectively. These data correlated well with the development of a normal-sized thymus, as evaluated by ultrasonography (in Patients 1, 2, 4, and 5) and by magnetic resonance imaging in Patient 5 (respective size at one year or more, 23 by 15 by 11.5 mm, 21 by 13 by 10 mm, 27 by 34 by 13 mm, and 19 by 15 by 7 mm) (Fig. 3C).

Expression of 17 $V\beta$ families of T-cell receptors in

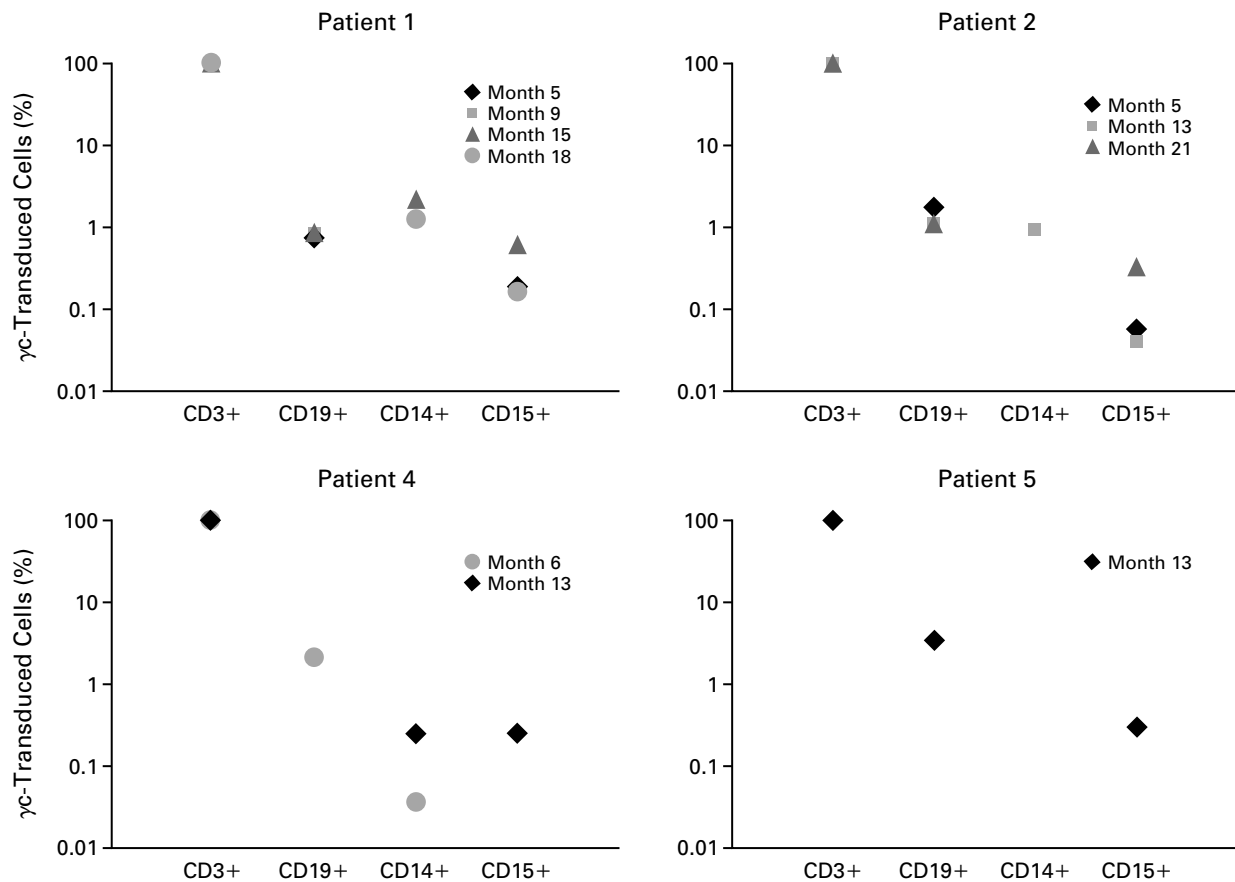


Figure 2. Frequency of Sorted T Cells (CD3+), B Cells (CD19+), Monocytes (CD14+), and Granulocytes (CD15+) Containing the Common γ (γ c) Chain after Gene Therapy in Patients 1, 2, 4, and 5. Real-time quantitative polymerase-chain-reaction analysis of DNA was used to determine the frequency of vector-containing cells, as described in the Methods section.

Patients 1, 2, 4, and 5 was similar to that in age-matched controls, and in these patients CD4+ and CD8+ T-cell populations remained stable. In all patients, a gaussian distribution of the lengths of complementarity-determining region 3 for 22 tested Vβ families of T-cell receptors was observed (see Supplementary Appendix 1).

Capacity for T-Cell Proliferation

At the last follow-up visit, T cells from Patients 1, 2, 4, and 5 exhibited normal proliferative responses to in vitro stimulation with phytohemagglutinin and anti-CD3 antibody (see Supplementary Appendix 1). Antigen-specific proliferative T-cell responses were also observed after immunization of those four patients with tetanus toxoid and polioviruses (see Supplementary Appendix 1). The addition of interleukin-2 to T cells from Patients 4 and 5 enhanced in vitro proliferative responses to tetanus toxoid. T cells from Patient 1, who was immunized with bacille Calmette–Guérin at two months of age, also had a proliferative response to tuberculin (purified protein derivative).

Development of Natural Killer Cells

Natural killer cells became detectable 15 to 45 days after gene therapy in Patients 2, 4, and 5 and 150 days after gene therapy in Patient 1 (Fig. 4). In Patients 2 and 4, and to a lesser magnitude in Patient 5, the levels of natural killer cells peaked two to four months after gene therapy and then gradually decreased. In Patient 3, natural killer cells were also detected in the blood beginning on day 45. These cells expressed γc as detected by immunofluorescence analysis (see Supplementary Appendix 1) and exhibited cytotoxic activity against K562 target cells (data not shown).

Serum Immunoglobulins and Antibody Production

Serum IgG, IgA, and IgM levels at 25, 21, and 13 months in Patients 1, 2, and 5, respectively, were

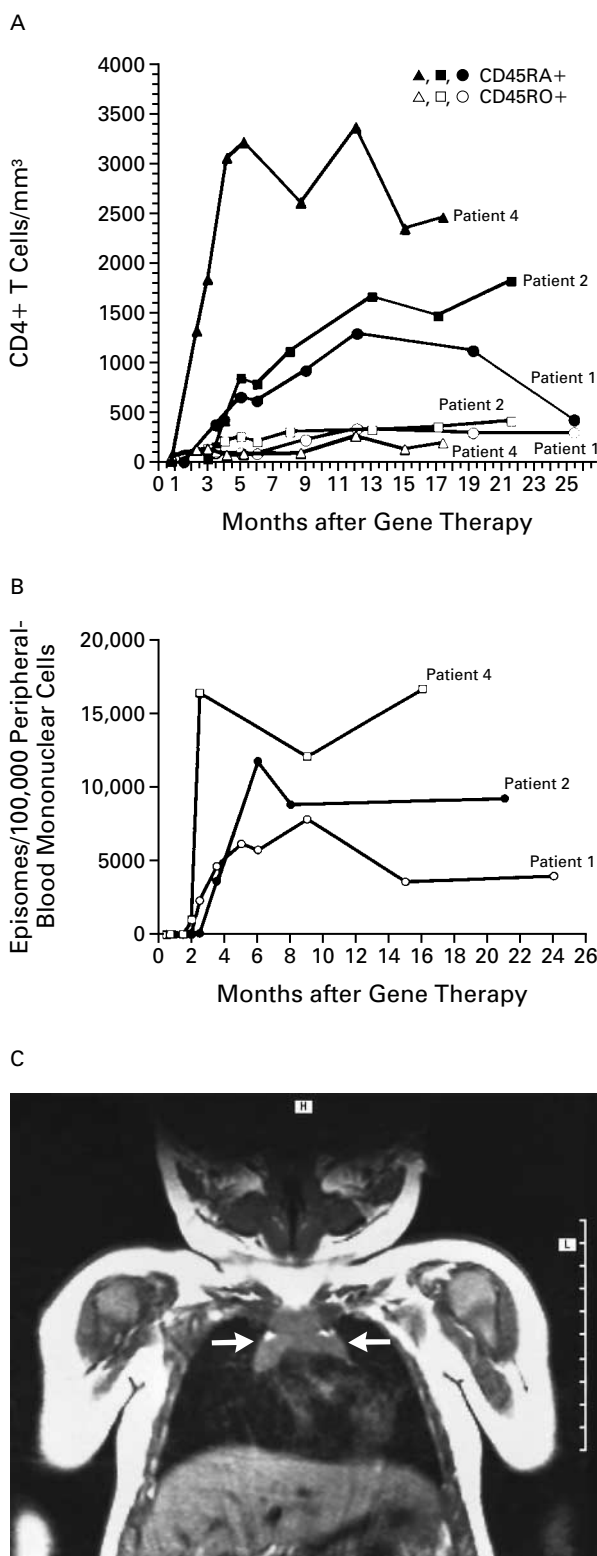


Figure 3. Numbers of Naive (CD45RA+) and Memory (CD45RO+) T Cells (Panel A) and Numbers of T-Cell Antigen-Receptor Episomes (Panel B) after Gene Therapy in Patients 1, 2, and 4 and Magnetic Resonance Image of a Coronal Section of the Thymus in Patient 5 Five Months after Gene Therapy (Panel C).

In Panel A, phenotypic quantification of naive and memory CD4+ T cells was performed with the use of double staining with fluorochrome-conjugated antibodies against CD4 and CD45RA or CD45RO. In Panel B, numbers of T-cell antigen-receptor episomes in peripheral-blood mononuclear cells were evaluated at different times. The normal range of T-cell antigen-receptor episomes for age-matched controls is 2500 to 20,000 per 100,000 peripheral-blood mononuclear cells. Arrows in Panel C show a normal-sized thymus after reconstitution of T cells.

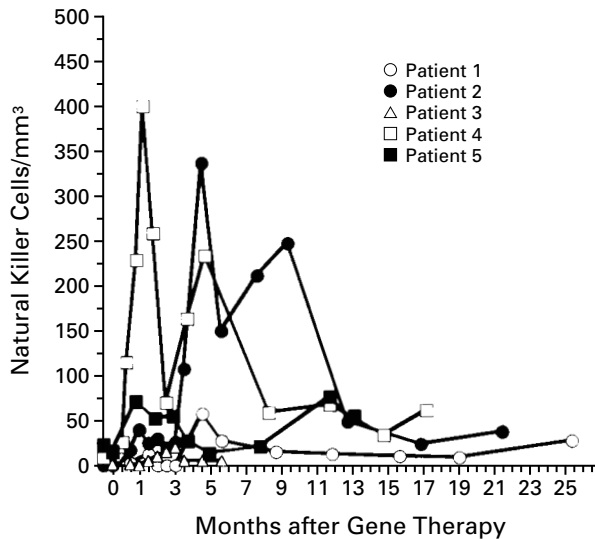


Figure 4. Absolute Numbers of CD56+ and CD16+ Cells per Cubic Millimeter of Whole Blood after Gene Therapy in Patients 1 through 5.

within the age-related normal range (Fig. 5). Low IgG and IgA levels persisted in Patient 4 (Fig. 5). Antibodies against tetanus toxoid, diphtheria toxoid, and poliovirus antigens were first found one month after the third immunization (Table 2) and persisted for more than six months in Patients 1, 2, and 4. Antibodies against *S. pneumoniae* in Patient 2 and *H. influenzae* in Patient 1 and Patient 2 were also detected. In contrast, immunization of Patient 5 failed to elicit an antibody response. Isohemagglutinins were consistently detected in the serum of Patients 1, 2, and 4 one year or more after gene therapy (Table 2). In three patients, the percentage of CD27+ and CD19+ B cells was similar to that of age-matched controls (see Supplementary Appendix 1).

Integration and Expression of γ_c Provirus

In Patients 1, 2, 4, and 5, all CD3+ T cells carried the γ_c transgene, as compared with 1 to 5 percent of B cells, 0.05 to 2 percent of monocytes, and 0.05 to 0.5 percent of granulocytes (Fig. 2). The frequency of γ_c -containing T cells, B cells, monocytes, and granulocytes was stable during the study period (Fig. 2). In Patients 2, 4, and 5, the presence of the γ_c gene coincided with the expression of γ_c chains (see Supplementary Appendix 1). In bone marrow samples obtained from Patient 2 and Patient 4 21 and 13 months, respectively, after gene transfer, 1 to 5 percent of colony-forming units derived from cultured CD34+ cells contained the transgene (frequency of

long-term-culture initiating cells, 1:1000 in Patient 2 and 1:500 in Patient 4) (data not shown).

Patient 3

Reconstitution of T cells failed to occur in Patient 3 (Fig. 1), despite the presence of γ_c -positive cells, as detected by PCR and immunofluorescence analysis of peripheral-blood mononuclear cells from day 30 up to four months after gene transfer. After splenectomy, a strong γ_c signal was detected among sorted CD19+ and CD16+ cells by nonquantitative PCR analysis. There were no CD3+ T cells in the spleen, and provirus (i.e., vector) was not detected in a bone marrow sample obtained at the time of splenectomy.

DISCUSSION

We found that four of five patients with X-linked severe combined immunodeficiency due to a deficiency of the γ_c chain who were treated with autologous CD34+ cells from bone marrow that had been transduced ex vivo with the γ_c gene showed evidence of a functional immune system and sustained clinical benefit. These results extend a preliminary report of two patients treated in this way.⁷ The gene-therapy protocol we used is safe, and no evidence of the emergence of a replication-competent retrovirus has been detected.

The evidence that virtually all T cells and natural killer cells but fewer B cells and myeloid cells were transduced suggests that γ_c expression gives progenitors of T cells and natural killer cells a selective growth advantage. Since transduced monocytes, granulocytes, and colonies derived from long-term cultures of transduced CD34+ cells were consistently detected one to two years after gene transfer, it is likely that long-lived immature progenitor cells were targeted by the vector. Moreover, the persistence of T-cell antigen-receptor episomes,^{11,12} naive T cells, and the development of a normal-sized thymus indicate ongoing formation of T cells and thymopoiesis, which most likely originated from transduced CD34+ progenitors. These findings suggest that both committed myeloid and lymphoid progenitor cells were transduced (implying that these cells persist in the bone marrow for at least one to two years) or that uncommitted pluripotent progenitor cells were transduced by the γ_c -containing vector. Evaluation of provirus integration sites in myeloid and lymphoid cells^{14,15} should help clarify this issue.

In our four successfully treated patients, the pattern of restoration of T cells differed from that observed after transplantation of haploidentical hematopoietic stem cells in patients with severe combined immunodeficiency.^{3,4} After the latter, T cells usually begin to appear within four to six months, and the

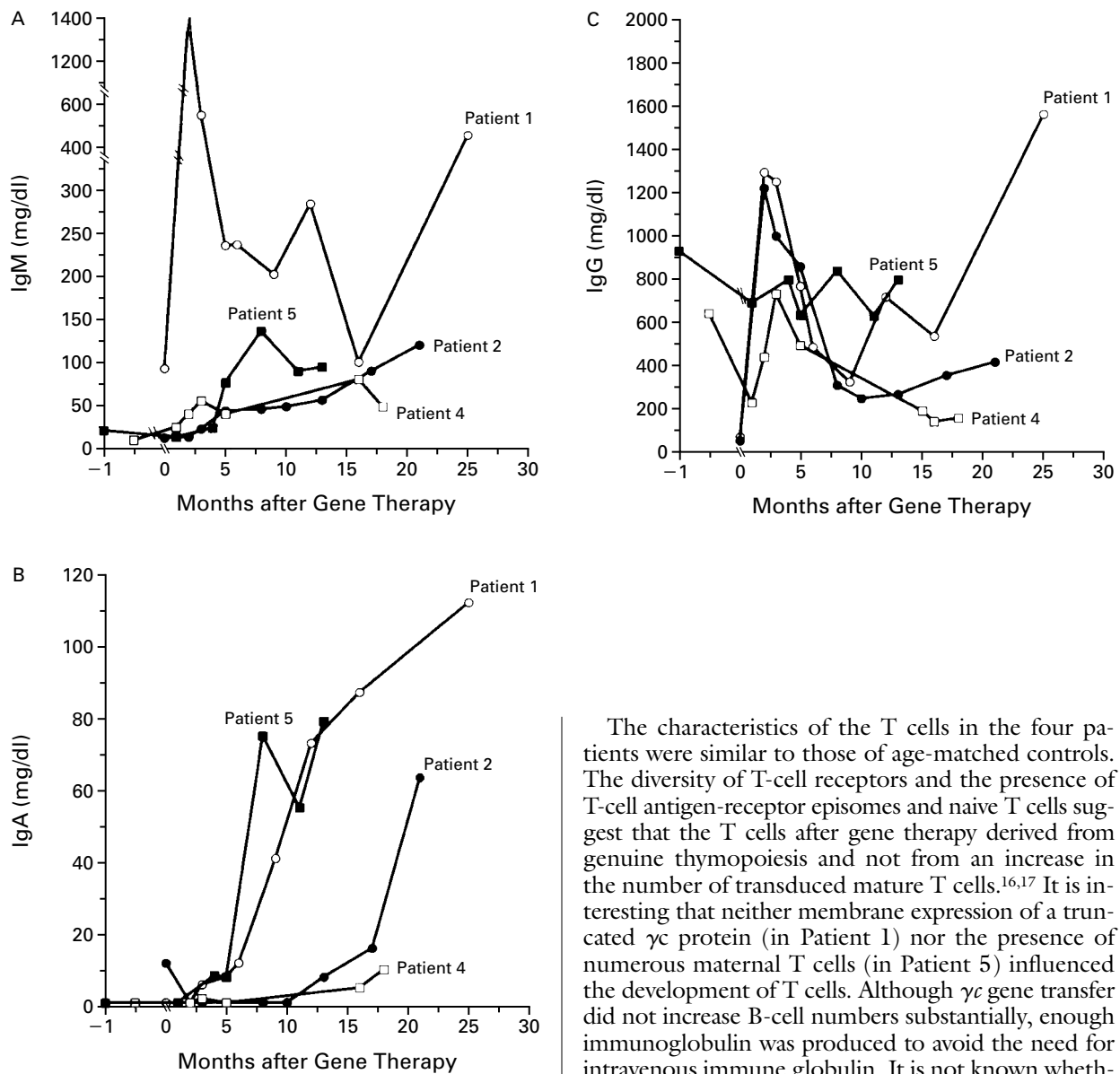


Figure 5. Serum Levels of IgM (Panel A), IgA (Panel B), and IgG (Panel C) after Gene Therapy in Patients 1, 2, 4, and 5. In Patient 1, the peak level of monoclonal IgM occurred two months after gene therapy.

number of T cells in peripheral blood rarely exceeds 2000 per cubic millimeter.^{3,4} In contrast, after gene therapy, T cells appeared within two to four months, at levels of 2000 to 8000 per cubic millimeter. The absence of graft-versus-host disease and the ex vivo activation of CD34+ cells with cytokines could have contributed to the rapid reconstitution.

The characteristics of the T cells in the four patients were similar to those of age-matched controls. The diversity of T-cell receptors and the presence of T-cell antigen-receptor episomes and naive T cells suggest that the T cells after gene therapy derived from genuine thymopoiesis and not from an increase in the number of transduced mature T cells.^{16,17} It is interesting that neither membrane expression of a truncated γ_c protein (in Patient 1) nor the presence of numerous maternal T cells (in Patient 5) influenced the development of T cells. Although γ_c gene transfer did not increase B-cell numbers substantially, enough immunoglobulin was produced to avoid the need for intravenous immune globulin. It is not known whether the few transduced B cells account for the production of antibodies in these patients or whether nontransduced B cells are also involved.¹⁸ Since there were more detectable memory B cells (CD27+ and CD19+) than transduced B cells, it is possible that γ_c -negative B cells retain some function.

In conclusion, our study demonstrates that the infusion of autologous γ_c -transduced cells, despite the low efficiency of the transduction process, can repair the immune system in patients with X-linked severe combined immunodeficiency. Although the repair is incomplete, it is sufficient to provide protective immunity. Despite an obvious requirement for long-term assessment and further analysis in a larger cohort of patients, these results suggest that a similar approach

TABLE 2. PEAK ANTIBODY RESPONSES AFTER IMMUNIZATION.*

ANTIBODY ASSAY	PATIENT 1	PATIENT 2	PATIENT 4	PATIENT 5	CONTROLS
Diphtheria toxoid (IU/ml)	3	93	22	<0.1	>0.10
Tetanus toxoid (IU/ml)	3	63	89	<0.1	>0.10
Poliovirus titer					
First	1:640	1:640	1:20	0	>1:40
Second	1:320	1:640	1:80	1:20	>1:40
Third	1:160	1:160	1:40	0	>1:0
Anti-A antibody titer	1:64	1:32	1:8	1:4	>1:8
Anti-B antibody titer	1:32	—	—	—	—
<i>Haemophilus influenzae</i> (%)†	26	16	ND	ND	>10
<i>Streptococcus pneumoniae</i> (μg/ml)	ND	8	ND	ND	>0.3

*Patients were immunized three times with diphtheria toxoid, tetanus toxoid, and poliovirus between month 4 and month 6; they were immunized with *Streptococcus pneumoniae* and *Haemophilus influenzae* one year after gene therapy. Serum antibodies were measured in serum samples drawn every three months thereafter. ND denotes not done.

†A positive value is more than 10 percent.

could be used for other forms of severe combined immunodeficiency.¹⁹⁻²⁴

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REFERENCES

- Noguchi M, Nakamura Y, Russell SM, et al. Interleukin-2 receptor gamma chain: a functional component of the interleukin-7 receptor. *Science* 1993;262:1877-80.
- Sugamura K, Asao H, Kondo M, et al. The common gamma-chain for multiple cytokine receptors. *Adv Immunol* 1995;59:225-77.
- Buckley RH, Schiff SE, Schiff RI, et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med* 1999;340:508-16.
- Haddad E, Landais P, Friedrich W, et al. Long-term immune reconstitution and outcome after HLA-nonidentical T-cell-depleted bone marrow transplantation for severe combined immunodeficiency: a European retrospective study of 116 patients. *Blood* 1998;91:3646-53.
- Haddad E, Le Deist F, Aucouturier P, et al. Long-term chimerism and B-cell function after bone marrow transplantation in patients with severe combined immunodeficiency with B cells: a single-center study of 22 patients. *Blood* 1999;94:2923-30.
- Patel DD, Gooding ME, Parrott RE, Curtis KM, Haynes BF, Buckley RH. Thymic function after hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med* 2000;342:1325-32.
- Cavazzana-Calvo M, Haccin-Bey S, de Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000;288:669-72.
- LeDeist F, Hivroz C, Partiseti M, et al. A primary T-cell immunodeficiency associated with defective transmembrane calcium influx. *Blood* 1995;85:1053-62.
- Pannetier C, Cochet M, Darche S, Casrouge A, Zoller M, Kourilsky P. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments. *Proc Natl Acad Sci U S A* 1993;90:4319-23.
- Towers GJ, Stockholm D, Labrousse-Najburg V, Carlier F, Danos O, Pages JC. One step screening of retroviral producer clones by real time quantitative PCR. *J Gene Med* 1999;1:352-9.
- Douek DC, McFarland RD, Keiser PH, et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature* 1998;396:690-5.
- Kong FK, Chen CL, Six A, Hockett RD, Cooper MD. T cell receptor gene deletion circles identify recent thymic emigrants in the peripheral T cell pool. *Proc Natl Acad Sci U S A* 1999;96:1536-40.
- Issaad C, Croisille L, Katz A, Vainchenker W, Coulombel L. A murine stromal cell line allows the proliferation of very primitive human CD34+/CD38- progenitor cells in long-term cultures and semisolid assays. *Blood* 1993;81:2916-24.
- Hanazono Y, Brown KE, Handa A, et al. In vivo marking of rhesus monkey lymphocytes by adeno-associated viral vectors: direct comparison with retroviral vectors. *Blood* 1999;94:2263-70.
- Schmidt M, Hoffmann G, Wissler M, et al. Detection and direct genomic sequencing of multiple rare unknown flanking DNA in highly complex samples. *Hum Gene Ther* 2001;12:743-9.
- Mackall CL, Bare CV, Granger LA, Sharrow SO, Titus JA, Gress RE. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. *J Immunol* 1996;156:4609-16.
- Berzins SP, Boyd RL, Miller JE. The role of the thymus and recent thymic migrants in the maintenance of the adult peripheral lymphocyte pool. *J Exp Med* 1998;187:1839-48.
- White H, Thrasher A, Veys P, Kinnon C, Gaspar HB. Intrinsic defects of B cell function in X-linked severe combined immunodeficiency. *Eur J Immunol* 2000;30:732-7.
- Macchi P, Villa A, Giliani S, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature* 1995;377:65-8.
- Bunting KD, Sangster MY, Ihle JN, Sorrentino BP. Restoration of lymphocyte function in Janus kinase 3-deficient mice by retroviral-mediated gene transfer. *Nat Med* 1998;4:58-64.
- Puel A, Ziegler SE, Buckley RH, Leonard WJ. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. *Nat Genet* 1998;20:394-7.

- 22.** Schwarz K, Gauss GH, Ludwig L, et al. RAG mutations in human B cell-negative SCID. *Science* 1996;274:97-9.
- 23.** Moshous D, Callebaut I, de Chasseval R, et al. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 2001;105:177-86.

- 24.** Kung C, Pingel JT, Heikinheimo M, et al. Mutations in the tyrosine phosphatase CD45 gene in a child with severe combined immunodeficiency disease. *Nat Med* 2000;6:343-5.

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CORRECTION

Gene Therapy for Severe Combined Immunodeficiency Disease

To the Editor: Correction of X-linked severe combined immunodeficiency by infusion of autologous CD34+ stem cells transduced with retrovirus containing common γ chain, reported by Hacein-Bey-Abina et al. (April 18 issue),¹ is a milestone in medicine. We used a different therapy with a similarly good outcome.

X-linked severe combined immunodeficiency was diagnosed in two patients after the initiation of mechanical ventilation for pulmonary failure caused by infections. Immediately after the diagnosis had been made, haploidentical CD34+ peripheral progenitor cells mobilized with granulocyte colony-stimulating factor were isolated to a purity of more than 99 percent.² These cells were infused with no preparative regimen and no prophylaxis against graft-versus-host disease. Both patients showed signs of T-cell reconstitution beginning three weeks after the CD34+ infusion and were weaned from the ventilator. They are in excellent health, without graft-versus-host disease, 34 and 68 months after transplantation. Patient 1 does not need replacement immune globulin. Patient 2 received a "booster" infusion of CD34+ stem cells from the original donor one year later to improve B-cell function and now receives immune globulin every three months.

Our experience indicates that purified haploidentical CD34+ progenitor cells reconstitute the T-cell compartment and can correct the B-cell defect. Given the possibility of long-term risks^{3,4} and the availability of effective alternatives, we think that broader application of gene therapy for the treatment of patients with severe combined immunodeficiency or strategies for the correction of persistent B-cell deficiency after successful allogeneic transplantation⁵ are premature and warrant longer follow-up.

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References

- Hacein-Bey-Abina S, Le Deist F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J Med* 2002;346:1185-1193.
- Schumm M, Lang P, Taylor G, et al. Isolation of highly purified autologous and allogeneic peripheral CD34+ cells using the CliniMACS device. *J Hematother* 1999;8:209-218.
- Donahue RE, Kessler SW, Bodine D, et al. Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer. *J Exp Med* 1992;176:1125-1135.
- Li Z, Dullmann J, Schiedmeier B, et al. Murine leukemia induced by retroviral gene marking. *Science* 2002;296:497-497.
- Rosen FS. Successful gene therapy for severe combined immunodeficiency. *N Engl J Med* 2002;346:1241-1243.

The authors reply:

To the Editor: Handgretinger et al. describe two cases of successful haploidentical hematopoietic stem-cell transplantation for X-linked severe combined immunodeficiency. It is indeed known that partially compatible hematopoietic stem-cell transplantation can provide T-cell reconstitution in 70 to 80 percent of cases.^{1,2} Nevertheless, haploidentical hematopoietic stem-cell transplantation has a number of pitfalls. Despite low numbers of T cells in the graft, graft-versus-host disease does develop in some cases (5 to 10 percent). T-cell repopulation after haploidentical hematopoietic stem-cell transplantation is slow.^{1,2} A period of more than three months is usually required before T cells can be detected. More important, Patel et al. have reported that after the performance of haploidentical hematopoietic stem-cell transplantation without myeloablation, T-cell immunity declines over time.³ Finally, correction of B-lymphocyte immunity is infrequent in patients with X-linked severe combined immunodeficiency who undergo haploidentical hematopoietic stem-cell transplantation in the absence of myeloablation.^{1,4} In contrast, so far all patients who have received gene therapy, with a follow-up of more than one year, in whom T-cell immunity has developed do not require intravenous immune globulin therapy. These observations justify further assessment of gene therapy as an alternative to hematopoietic stem-cell transplantation.

The potential risk of gene therapy must not be underestimated and must be balanced against the risk of alternative therapy. The concern of Handgretinger et al. is not entirely appropriate, since helper virus and the expression of a membrane receptor, which accounted for reported toxic effects, are irrelevant to our trial. In our opinion, gene therapy can be considered an option worth exploring for patients with severe combined immunodeficiency.

We would also like to note that on page 1185 of our article, author Lily Leiva's name was misspelled, and the affiliation for Dr. Leiva and author Ricardo Sorensen should have included both Louisiana State University Health Sciences Center and Children's Hospital, New Orleans.

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References

1. Buckley RH, Schiff SE, Schiff RI, et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med* 1999;340:508-516.
2. Haddad E, Landais P, Friedrich W, et al. Long-term immune reconstitution and outcome after HLA-nonidentical T-cell-depleted bone marrow transplantation for severe combined immunodeficiency: a European retrospective study of 116 patients. *Blood* 1998;91:3646-3653.
3. Patel DD, Gooding ME, Parrott RE, Curtis KM, Haynes BF, Buckley RH. Thymic function after hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med* 2000;342:1325-1332.
4. Haddad E, Le Deist F, Aucouturier P, et al. Long-term chimerism and B-cell function after bone marrow transplantation in patients with severe combined immunodeficiency with B cells: a single-center study of 22 patients. *Blood* 1999;94:2923-2930.