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A murine model for B-lymphocyte somatic cell gene therapy

(retroviral vectors/adenosine deaminase/SCID mice/lymphocyte homing)

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ABSTRACT **Mature primary B lymphocytes represent a potentially important cellular target for somatic cell gene therapy, which could prove advantageous for the treatment of certain metabolic and immunologic disorders. Their capacity to serve as antigen-presenting cells could be utilized for triggering and/or potentiating immune responses to tumors and viruses. Alternatively, B cells expressing an autoantigen could be manipulated to induce antigen-specific unresponsiveness for treatment of autoimmune diseases. Efficient expression of an exogenous gene product in long-lived B lymphocytes could be particularly useful for providing a corrected gene product in the bloodstream. Despite these advantages, efficient gene transfer into mature primary B cells has not been reported. One reason for this is that current protocols for retroviral vector-mediated gene transfer into lymphocytes rely on *in vitro* expansion and/or drug selection. This precludes the use of mature primary B cells as targets, since they cannot be readily cultured for long periods of time. In this report, we describe an efficient and rapid protocol for the introduction of exogenous genes into primary B cells without the need for drug selection. We have used retroviral vectors containing the human adenosine deaminase gene as a marker gene, since the biological activity of this enzyme is easy to measure and is readily distinguishable from that of the endogenous mouse adenosine deaminase. Upon adoptive transfer into SCID mice, infected B cells continuously expressing one to three copies of the human adenosine deaminase gene could be found in the spleens of recipient animals for at least 3 months.**

The efficient transfer of exogenous genes into primary B lymphocytes has direct therapeutic potential for the treatment of diseases that affect the B-cell compartment, such as X-linked agammaglobulinemia (1, 2) and adenosine deaminase (ADA) deficiency (3, 4). It also has therapeutic potential for genetic diseases in which delivery of a normal gene product into the bloodstream can be helpful, such as hemophilia (factor VIII and factor IX deficiency) (5–7) and lipoprotein lipase deficiency (8, 9). Furthermore, since B cells function as antigen-presenting cells, they can be manipulated to express tumor or viral antigens to initiate or augment anti-tumor or anti-viral immune responses. Conversely, the adoptive transfer of B cells that constitutively express an autoantigen, combined with signals for the downregulation of accessory signaling molecules, could be used to induce antigen-specific unresponsiveness (10, 11). This approach could be useful for the treatment of T-cell-mediated autoimmune diseases by the induction of peripheral tolerance. An additional advantage of using B cells as target cells for gene therapy is that much is known about the regulation of expression of immunoglobulin heavy- and light-chain genes. This information could be used to optimize tissue-specific expression and secretion of exogenous genes in B cells. Moreover, the immunoglobulin secretory pathway could be utilized for the secretion of large quantities of an exogenous gene product.

A number of gene-therapy protocols involving retroviral vector-mediated transfer of exogenous genes into lymphocytes are currently in clinical trials. These include the introduction of the *ADA* gene into peripheral blood T cells of ADA-deficient, severe combined immunodeficiency (SCID) patients (12), and the introduction of marker genes or lymphokine genes into tumor-infiltrating T cells (13, 14). These protocols rely on long-term *in vitro* expansion and/or drug selection of the target cells to enrich for stably infected cells. Since B cells cannot be maintained *in vitro* for long periods of time, these procedures are inadequate for the introduction of genes into B cells. More importantly, cells from long-term cultures do not home properly to lymphoid organs upon adoptive transfer (15–17).

In this report we describe an animal model system for somatic cell gene therapy using primary B lymphocytes, involving an efficient gene-transfer protocol that obviates the need for *in vitro* drug selection. In this model, within 40 hr, we can reproducibly infect spleen and lymph node (LN) B cells with a retroviral vector containing the human *ADA* gene expressed from its own promoter. We chose to use the human *ADA* gene as a marker, since the human and mouse ADA enzymes are readily distinguished by electrophoretic mobility, and enzyme levels can be determined by a simple assay. The target cells contain on average one to three copies of the provirus per cell; they express high levels of human ADA; and when adoptively transferred into SCID mouse hosts, they home to lymphoid organs and persist for at least 3 months without any detectable loss in the level of expression of the introduced gene.

MATERIALS AND METHODS

Mice. BALB/cBy SCID male and female mice were purchased from The Jackson Laboratory, and maintained in a germ-free environment. C.B-17 male and female mice were purchased from Taconic Farms.

Retroviral Vector and Virus-Producing Cell Line. AsADA is a Moloney murine leukemia virus (Mo-MLV)-based retroviral vector of the G1Na type (18), containing the neomycin phosphotransferase II (*neo*) gene, which is expressed from the long terminal repeat (LTR) promoter, and the human *ADA* gene, expressed from its endogenous promoter. This vector was kindly provided by Genetic Therapy, Inc. (Gaithersburg, MD). The GP+E-86 cell line is an NIH 3T3-based

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Abbreviations: ADA, adenosine deaminase; SCID, severe combined immunodeficiency; *neo*, neomycin phosphotransferase II gene; LN, lymph node(s); LPS, lipopolysaccharide; *GPT*, xanthine guanine phosphoribosyltransferase gene; LTR, long terminal repeat; Mo-MLV, Moloney murine leukemia virus.

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ecotropic murine packaging cell line (19). GP+E-86 cells were transfected with 5 μg of vector plasmid DNA by the Polybrene/dimethyl sulfoxide shock method (20), followed by selection for *neo* (with G418, 0.35 mg/ml) and for the xanthine guanine phosphoribosyltransferase (*GPT*) gene (xanthine, 0.25 mg/ml; mycophenolic acid, 25 μg/ml; hypoxanthine, 15 μg/ml). AsADA-transfected cells yielded virus stocks of 1.5×10^7 colony-forming units per ml, quantitated by inoculation of NIH 3T3 cells with serial dilutions of helper-cell supernatant, followed by selection with G418 for 2 weeks, at which time colonies were counted. AsADA producer cells were regularly tested and always found to be negative for production of replication-competent virus. Screening for replication-competent virus was performed by assaying for reverse transcriptase activity after a 2-week NIH 3T3 amplification step (21). We have also performed the more sensitive helper rescue assay (21) after NIH 3T3 amplification, using a BAG vector-transfected NIH 3T3 cell line which has undergone selection with G418. The BAG vector contains the *lacZ* gene and the *neo* gene (22). No colonies were detected by staining with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (23).

Preparation of Splenic and LN B Lymphocytes. Spleen and LN were obtained from 6- to 9-week-old mice. Enriched populations of B lymphocytes were prepared by depletion of T cells using the monoclonal antibody J1j, which is a rat anti-mouse Thy-1.2 antibody (24), and complement. This procedure routinely yields >97% B cells (from LN) and >85% B cells (from spleen) as analyzed by flow cytometry. Fewer than 0.5% T cells were detected in B-cell-enriched populations (data not shown). B cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and gentamicin (50 μg/ml) and were stimulated with lipopolysaccharide (LPS, 50 μg/ml) 16 hr before infection.

Gene Transfer. Gene transfer into primary B cells was performed by cocultivation with virus-producing cells as described (25). In brief, 10×10^6 stimulated B cells were plated in 10 ml of RPMI 1640 medium onto a confluent lawn of irradiated (1600 rads; 1 rad = 0.01 Gy) virus-producing cells in 100-mm tissue culture plates. Polybrene (6 μg/ml) and LPS (50 μg/ml) were added to the medium. B cells were harvested 24 hr later and either were adoptively transferred to lightly irradiated (200 rads) BALB/cBy SCID mice for *in vivo* studies ($5–25 \times 10^6$ cells per mouse i.v. into the tail vein) or cultured for 48 hr in fresh medium containing LPS (50 μg/ml) for *in vitro* studies, at which time target cell genomic DNA was extracted for Southern blotting analysis and protein extracts were prepared for ADA activity assays.

Southern Blot Analysis. Southern blotting with *Sac* I-digested genomic DNA (10 μg per lane) was performed according to standard methods (26), using a ^{32}P-labeled *neo*-specific probe. The probe was labeled by random priming. Controls for copy number were made by digesting pAsADA plasmid DNA with *Sac* I and loading amounts corresponding to 0.5, 1, and 5 proviral copies per genome on the gel.

ADA Assay. Lymphocytes (1×10^6) were lysed by repeated freezing and thawing, and the lysate was applied to a cellulose acetate plate (Helena Laboratories). Human and murine ADA enzymes were separated by electrophoresis on cellulose acetate plates (27). Enzyme activity was detected by reaction of the separated ADA enzymes on the plate with an agar overlay containing adenosine (2 mg/ml) (Sigma), nucleoside phosphorylase (15 μg/ml) (Boehringer Mannheim), xanthine oxidase (0.06 unit/ml) (Boehringer Mannheim), phenazine methosulfate (0.01 mg/ml) (Sigma), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.1 mg/ml) (Sigma) in phosphate buffer at 37°C for 20 min in the dark.

Homing of B Cells. C.B-17 splenic B cells (treated with anti-Thy-1.2 plus complement) were stimulated with LPS for 16 hr, cocultivated for 24 hr with irradiated AsADA producer cells, and then labeled with ^{51}Cr. Live cells were purified on a one-step density gradient (12% Ficoll, Pharmacia, and 9.6% sodium diatrizoate, Sigma) and were then labeled in RPMI 1640 with a low concentration of ^{51}Cr (as Na_2CrO_4), 100 μCi (1 μCi = 37 kBq) per 15×10^6 cells, for 1 hr at 37°C. As a control, freshly obtained splenic B cells (treated with anti-Thy-1.2 plus complement) were simultaneously labeled with ^{51}Cr. Five million labeled cells were injected i.v. into three BALB/cBy SCID mice per group. Eighteen hours after injection, spleen, liver, gut, legs, and lungs were collected from each mouse and radioactivity was measured in a γ counter.

Flow Cytometric Analysis. Spleen cells from recipient SCID mice were analyzed by flow cytometry 1 or 3 months after gene transfer. Cells (1×10^6) were stained with monoclonal antibodies specific for the donor C.B-17 *Igh* allotype δ^b [Ig(5b)6.3] and the recipient BALB/cBy *Igh* allotype δ^a [Ig(5a)7.2] (28). Five thousand cells were analyzed on an Epics Profile (Coulter).

Semiquantitative PCR. PCR primers (5′-CTGGGTATTCGTCATGTCGA-3′ and 5′-GGCGGACTAGCGTCGAGGTT-3′) were made for amplification of a 478-bp sequence from the *GPT* gene present in the GP+E-86 packaging cell line (19) but not in the AsADA retroviral vector. As controls, to determine the specificity of the reaction, genomic DNA extracted from C.B-17 spleen cells was mixed with various amounts of GP+E-86 helper-cell DNA. PCR used 40 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min; 0.2 μM each primer; 0.2 mM each deoxynucleotide; 2 mM $MgCl_2$ in 70 mM Tris·HCl, pH 8.8/0.1% Triton X-100; and 2 units of *Taq* DNA polymerase. DNA (0.1 μg) from control mixtures or from the spleen of each SCID mouse adoptively transferred with AsADA-infected B cells was tested. Helper cell DNA diluted 1:4000 with spleen DNA could be detected by Southern blotting of one-tenth of the PCR product with a ^{32}P-labeled *GPT*-specific probe.

RESULTS

Efficient Gene Transfer into Splenic and LN B Cells. We used the Mo-MLV-based retroviral vector AsADA, containing the human *ADA* and the bacterial neomycin-resistance (*neo*) genes (Fig. 1*A*) to infect primary murine B cells. Splenic and LN B cells from C.B-17 mice were stimulated for 16 hr with LPS. Proliferation of the target cells is required for efficient integration of the provirus (29). B cells were then cocultivated with irradiated helper cells for 24 hr. With this method, we routinely obtained an infection efficiency of one to three proviral copies per target cell genome for both splenic and LN B cells (Fig. 1*B*, lanes 4 and 5). Copy number was assessed by Southern blotting of target-cell genomic DNA 48 hr after infection, using a probe specific for *neo* sequences present in the provirus, and compared with pAsADA plasmid DNA equivalent to 5, 1, or 0.5 provirus per genome (Fig. 1*B*, lanes 1–3). To rule out the possibility that part of the observed signal was due to contaminating helper cells that may have lifted during cocultivation, the supernatant from helper-cell plates that were manipulated as for infection but were not cocultivated with target cells was added to fresh LN cells at the time of genomic DNA extraction. The fresh LN cells served as a source of carrier DNA. No signal was detected in this control (Fig. 1*B*, lane 6).

In parallel experiments, the human ADA enzyme activity in both splenic and LN B cells was higher than the endogenous murine ADA activity (Fig. 1*C*, lanes 4 and 5). This is consistent with the high proviral copy number. AsADA producer cells, the human H-9 T-cell line, and uninfected murine LN cells served as standards for the human and

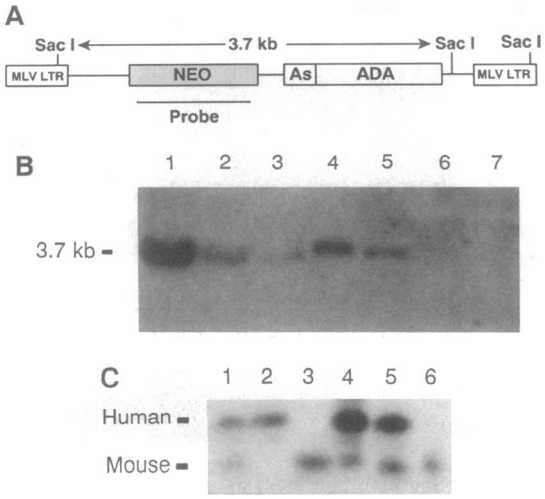

FIG. 1. (*A*) The retroviral vector AsADA. AsADA contains the bacterial *neo* gene expressed from the Mo-MLV LTR promoter and the human *ADA* gene expressed from its endogenous promoter. *Sac* I sites in the LTR yield a 3.7-kb fragment upon restriction enzyme digestion. (*B*) Southern blot analysis of splenic and LN B cells infected with AsADA. Primary C.B-17 spleen and LN B cells were stimulated with LPS and then were cocultivated with AsADA producer cells in the presence of LPS for 24 hr. Genomic DNA was extracted from the target cells 48 hr later, and Southern blotting was performed with the restriction enzyme *Sac* I and a *neo*-specific probe. Lanes: 1–3, *Sac* I-digested pAsADA plasmid DNA equivalent to 5, 1, and 0.5 proviral copy per cell, respectively; 4, AsADA-infected splenic B cells; 5, AsADA-infected LN B cells; 6, a supernatant control, performed by mixing uninfected LN cells with the supernatant from irradiated helper cells that had been plated as for cocultivation, at the time of genomic DNA extraction; 7, uninfected LN cells. (*C*) Assay of human ADA activity in murine B cell extracts. In parallel experiments, 48 hr after infection, the lysate from 1×10^6 target cells was electrophoresed on a cellulose acetate plate to separate human from murine ADA enzymes, and then ADA activity was detected by colorimetric enzyme assay. Lanes: 1, AsADA producer cells; 2, human standard: H-9 T-cell line; 3, murine standard: uninfected LN cells; 4, splenic B cells infected with AsADA; 5, LN B cells infected with AsADA; 6, a supernatant control (as in *B*).

murine enzymes (Fig. 1*C*, lanes 1–3). No signal resulting from potentially lifted helper cells was detected (Fig. 1*C*, lane 6).

Homing of ^{51}Cr-Labeled, AsADA-Infected B Cells in SCID Mice. A major problem that results from culturing primary cells for long times *in vitro* is that they do not home properly when introduced back into the recipient host (15–17). Since Southern blotting indicated that efficient gene transfer could be completed within 40 hr, without the need for an *in vitro* drug selection step, we postulated that the primary B cells would retain normal homing patterns *in vivo*. To test the homing patterns of C.B-17 splenic B cells infected with AsADA compared with fresh splenic B cells (anti Thy-1.2 plus complement-treated only), ^{51}Cr-labeled cells were transferred i.v. into groups of 3 *Igh*-congenic BALB/cBy SCID mice. Radioactivity in various organs was measured 18 hr later (Table 1). AsADA-infected splenic B cells exhibited homing patterns similar to fresh splenic B cells. In both groups, an appreciable number of cells homed to the spleen of recipient mice, consistent with published reports (30, 31), although the percentage of infected cells found in the spleen 18 hr after adoptive transfer, 7.14%, was lower than that of the control cells, 13.16%. Levels of radioactivity were highest in the liver in both groups, because the liver clears surface-altered lymphocytes. For infected cells, the spleen/liver ratio was 0.28, vs. 0.65 for freshly obtained B cells (anti-Thy-1.2-plus-complement treatment only), indicating

Table 1. Homing pattern of ^{51}Cr-labeled AsADA-infected splenic B cells injected i.v. into SCID mice

Organ(s)	Percent injected radioactivity*	
	Control	AsADA-infected
Spleen	13.2 ± 0.6	7.1 ± 0.6
Liver	20.2 ± 0.6	25.6 ± <0.1
Gut	2.0 ± 0.5	1.1 ± 0.7
Legs (bone marrow)	4.4 ± 0.6	1.9 ± 0.6
Lungs	2.0 ± 0.6	2.2 ± 0.6

Splenic B cells were stimulated with LPS for 16 hr and then cocultivated with AsADA producer cells for 24 hr prior to labeling with ^{51}Cr. Freshly obtained splenic B cells (control) were simultaneously labeled. Labeled cells (5×10^6) were injected into three mice for each group, and 18 hr later various organs were collected. Radioactivity was measured in a γ counter and is expressed as a percent of the injected radioactivity. Values give mean ± SD for three mice per group.

that the 40-hr cocultivation protocol reduced by ≈50% the number of cells that homed to the spleen. Although this must be taken into account, a significant proportion of AsADA-infected primary B cells retained the capacity to home to the spleen.

AsADA-Infected B Cells Persist Long-Term in the Spleen of SCID Mice. To assess whether AsADA-infected B cells persisted long-term *in vivo*, splenic and LN B cells from C.B-17 mice were infected with the AsADA vector virus and then adoptively transferred to 10 *Igh*-congenic BALB/cBy SCID mice ($5–25 \times 10^6$ cells per mouse, i.v.). Adoptively transferred B cells could be detected in the spleen of the recipient mice for at least 3 months. This was determined by flow cytometry using monoclonal antibodies specific for the donor type Igh-5 (δ) allotypic marker. Fig. 2*A* depicts flow cytometric analysis of the spleen of a representative SCID mouse adoptively transferred with LN B cells, 1 month after transfer: 24.3% of the lymphocytes in the spleen expressed the donor Igh-5 allotype and 16.8% expressed the host Igh-5 allotype. The host-type cells are most probably "leaky" cells that are found in most C.B-17 SCID mice (32). Fig. 2*B* depicts flow cytometric analysis of the spleen of a representative SCID mouse adoptively transferred with splenic B cells: 44.8% of the lymphocytes were of donor origin, and only

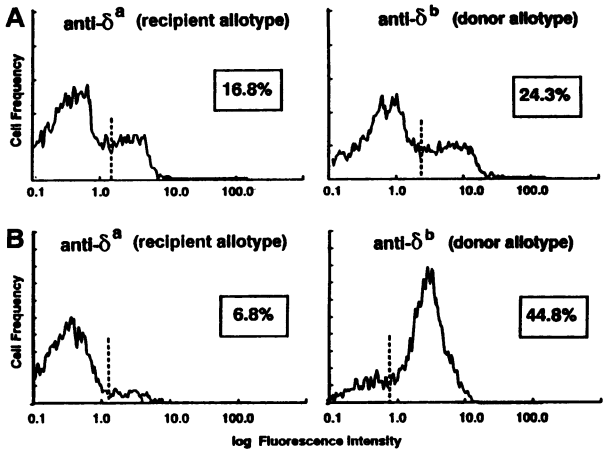

FIG. 2. Flow cytometric analysis of spleen cells from SCID mice reconstituted with AsADA-infected B cells. LN and splenic B cells from C.B-17 mice were infected with AsADA and injected into *Igh*-congenic BALB/cBy SCID mice. Spleen cells were analyzed by flow cytometry 1 month after transfer from a representative mouse injected with LN B cells (*A*) and 3 months after transfer from a representative mouse injected with splenic B cells (*B*). Cells (1×10^6) were stained with monoclonal antibodies specific for the donor Igh allotype δ^b and the recipient δ^a.

6.8% were of host origin 3 months after transfer. Donor-type lymphocytes were also detected in LN, however, in lower numbers (data not shown). This is consistent with published reports that mature B cells home preferentially to the spleen after adoptive transfer into mice (30, 31). The LN in SCID mice are atrophic and therefore very few of the adoptively transferred cells home to the LN (33).

Provirus could be detected for at least 3 months in the spleen and LN of recipient animals. Southern blotting of total genomic DNA isolated from whole spleen at 1 month and 3 months after transfer was positive for proviral sequences in 10 out of 10 mice tested. Fig. 3*A*, lanes 1–10, shows the proviral signals for the 10 mice at 1 or 3 months after adoptive transfer. Compared with AsADA plasmid-copy-number controls equivalent to 0.5, 1, and 5 provirus per cell (Fig. 3*A*, lanes 12–14, respectively), the provirus is present in each spleen at 0.1–0.5 copy per cell, depending on the number of nonlymphoid and leaky cells which are also present in each spleen. Only a small percentage of the cells in the spleens of the reconstituted SCID mice are lymphocytes, and therefore most of the DNA analyzed is derived from irrelevant, nonlymphoid cells.

Although the supernatant controls in Fig. 1 *B* and *C* indicated that contaminating helper cells did not contribute to the observed signals, semiquantitative PCR was performed to rule out this possibility. To this end, we used *GPT*-specific primers to amplify sequences unique to the producer cell line, but not present in the retroviral vector. After Southern blotting of the PCR products, eight of the spleen samples were negative for helper cell-specific sequences, whereas two yielded a signal corresponding to less than 1 helper cell in 4,000 spleen cells (data not shown). Since in each spleen one copy of the provirus was detected per 5–10 cells, we conclude that contaminating helper cells did not contribute to the signal obtained from any of the recipient SCID mice.

Human ADA Expression in the Spleen of SCID Mice 3 Months After Gene Transfer. Exogenous gene expression was demonstrated in all of the recipient mice both at 1 month and at 3 months posttransfer, by enzymatic assay for ADA activity in cell lysates made from whole spleen tissue. Fig. 3*B*, lanes 3–12, depicts human ADA activity in the mice represented in Fig. 3*A*. In SCID spleen, the human ADA activity does not appear as strong as the murine ADA activity because there are many other types of cells present in the spleen which express the endogenous ADA gene (cells of the myeloid lineage and erythrocytes). When this is taken into account, the level of expression of the exogenous gene correlates well with the percentage of donor lymphocytes in individual mice as quantitated by flow cytometry. (Fig. 2 represents flow cytometric analysis of two representative mice.) Expression of human ADA did not decrease over time between 1 month and 3 months after transfer (Fig. 3*B*).

Although it may appear from Fig. 3 that the level of human ADA in each mouse did not precisely correlate with the proviral copy number, this is misleading, since the results reflect differences between sample collection used in the ADA assay versus Southern blotting. For the ADA assay each lane represents the lysate of whole spleen cells containing 1×10^6 lymphocytes. The number of nonlymphoid host cells varied in each sample. Host cells did not dilute out the signal from the exogenous human ADA, but they did contribute to the larger murine ADA signal. On the other hand, in Southern blotting, the signal from exogenous DNA was diluted by irrelevant host cells, since the genomic DNA was extracted from whole spleens. Therefore, the measure of ADA activity is independent of the number of host cells but the Southern blotting is affected. This explains the apparent variation between Fig. 3 *A* and *B*. Lymphocytes expressing the human *ADA* gene were also found in LN (data not shown).

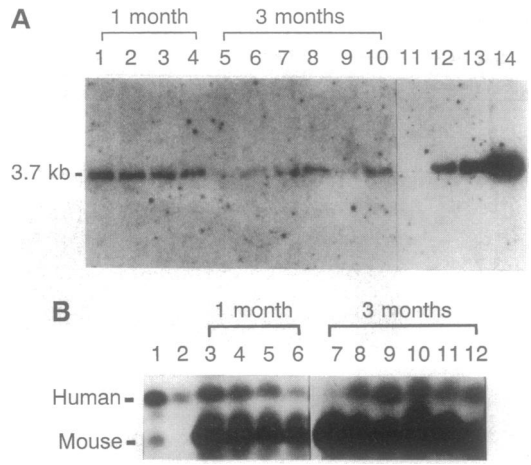

FIG. 3. (*A*) Southern blot analysis of genomic DNA from the spleen of SCID mice that were adoptively transferred with target B cells. Splenic B cells were infected with AsADA and injected immediately into 10 SCID mice ($5–25 \times 10^6$ cells per mouse, i.v.). Recipient mice were sacrificed either 1 or 3 months later, and genomic DNA was extracted from the spleens. Southern blotting was performed as in Fig. 1. Lanes: 1–4 and 5–10, spleens from recipient mice sacrificed 1 month and 3 months after transfer, respectively; 11, control spleen from a mouse that did not receive target cells; 12–14, *Sac* I-digested pAsADA plasmid DNA equivalent to 0.5, 1, and 5 proviral copies per cell, respectively. (*B*) Human ADA activity in SCID mice. Spleen cell lysates corresponding to 1×10^6 lymphocytes were assayed for human ADA activity as described in the legend to Fig. 1. Lanes: 1, AsADA helper cells; 2, human H-9 T-cell line; 3–6 and 7–12, spleens from recipient mice sacrificed 1 month and 3 months after transfer, respectively.

DISCUSSION

We describe a reproducible animal model system for the introduction and expression of exogenous genes into mature primary B cells. Our data suggest that the transduction of B cells offers a viable option for the long-term expression of an exogenous gene in the lymphoid compartment. This simple and relatively inexpensive animal model may prove appropriate for use as an initial screening procedure for various somatic cell gene-therapy protocols. For example, it could be particularly useful to test for potentially negative effects of an exogenous gene on the lymphoid compartment or other organs, such as the liver. Effects on the liver are especially important to assess, since a large proportion of transferred cells are cleared by the liver (Table 1).

The model presented here is practical for at least three reasons. (*i*) The infection protocol is very efficient, resulting in one to three copies per cell. An important consequence of efficient infection is the elimination of the need for drug selection to enrich for infected cells. This enables a short *in vitro* culture period, which results in better homing of the target B cells to lymphoid organs. (*ii*) A large proportion of splenic and LN B cells are long-lived (33, 34). This might reduce or even eliminate the need for repeated administration of infected cells. (*iii*) Expression of the introduced gene in mature primary B cells is very stable. In contrast, stable expression of genes introduced into bone marrow stem cells has been problematic (35, 36).

Our experiments demonstrate that the target cell population harbored one to three proviral copies per cell on average. From our experimental design we cannot determine directly the percentage of infected cells. Therefore, it could be argued that only 10% of the cells might harbor 10–30 copies of the introduced gene. This could only occur if the individual B cells in the target population have vastly different susceptibilities to infection. We think that this is highly unlikely since practically all of the target B cells proliferate in response to

LPS. Thus the majority of B cells should be equally susceptible to vector virus infection.

Mature primary B cells have not previously been used as targets for retroviral vector-mediated gene transfer. However, several laboratories have reported the successful introduction of exogenous genes into primary T lymphocytes, such as marker genes (for cell tagging) or lymphokine genes into tumor-infiltrating cells, or the *ADA* gene into peripheral blood lymphocytes (13, 37–39). In all of these cases, when drug selection was not used, the efficiency of infection was very low (5% at best). Studies in which the *ADA* gene was introduced into human peripheral blood lymphocytes taken from ADA-deficient patients indicated that the introduced *ADA* gene conferred a selective advantage on the corrected T cells when adoptively transferred into BNX immunodeficient mice (39, 40). This most likely occurs in the clinical situation. In our model, the introduction of the human *ADA* gene into murine B cells does not confer such selective pressure, because the survival of the target cells does not depend on the introduced gene. That we obtained a strong signal at both the DNA and protein levels *in vivo* suggests that the majority of the target B cells were infected and that the infection did not compromise long-term target cell survival. Although we used *ADA* as a marker gene in this murine system, for patients with SCID due to ADA deficiency, transfer of *ADA* into B cells might also prove useful, since these patients are deficient in both B cells and T cells.

Perhaps the most intriguing use of B cells as targets for somatic cell gene therapy is the potential for manipulating immune responses. The ability of activated B cells to present antigen could be exploited to potentiate immune responses to tumor or viral antigens. Alternatively, the adoptive transfer of resting B cells expressing self-antigens could be used to confer antigen-specific unresponsiveness, as has been shown in other experimental systems. For example, the transfer of Mls^a B cells into Mls^b mice was shown to induce clonal anergy and/or clonal deletion of host Mls^a-specific T cells (10, 11). This form of unresponsiveness is termed peripheral tolerance. The induction of peripheral tolerance could prove to be a useful approach for treating T-cell-mediated autoimmune diseases.

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