LacZ transgenic rats tolerant for β-galactosidase: recipients for gene transfer studies using lacZ as a reporter gene.

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Running title: LacZ transgenic rats tolerant for β-gal.
SUMMARY

Gene transfer of reporter genes may trigger immune responses against the heterologous protein resulting in shortening of gene expression and inflammation. We generated transgenic rats expressing the lacZ gene under the control of the HIV-1 LTR (HIV-lacZ) to obtain rats with undetectable transgene expression using histological methods, thus avoiding interference with β-gal expression from gene transfer, and displaying immune tolerance towards β-gal. LacZ transgenic mice with tolerance towards β-gal have already been used for gene transfer but rats constitute unique animal models with several advantages compared to mice. Two transgenic lines displayed low levels of β-gal mRNA in most organs tested, as detected only by RT-PCR. The protein was undetectable by immunohistology and was only detected in the thymus and spleen using a sensitive ELISA. HIV-lacZ transgenic rats displayed immune tolerance to β-gal since immunization with β-gal resulted in markedly lower cellular and antibody responses compared to wild-type controls whereas immunization with a non-related antigen, keyhole limpet hemocyanin (KLH), resulted in comparable immune responses. The usefulness of this model in gene transfer was tested using a retroviral vector, which do not elicit destructive immune responses against transduced cells. Retroviral-mediated nlslacZ gene transfer in the liver resulted in nuclear β-gal expression for >12 months in HIV-lacZ transgenic rats whereas wild-type controls showed nuclear β-gal expression for <1 month. After gene transfer of nlslacZ to the liver, antibodies, CTLs and proliferation against β-gal were detected in wild-type controls but not in HIV-lacZ transgenic rats. In conclusion, HIV-lacZ transgenic rats displaying low β-gal expression and immune tolerance towards β-gal are a useful tool to analyse the spatial and temporal expression of the β-gal protein in gene transfer experiments using lacZ as a reporter gene.
INTRODUCTION

The amelioration of gene therapy demands accurate measurement of gene transfer efficiency, inflammation and duration of gene expression for each vector type. Gene transfer of reporter genes is used to this end. However, most of these markers are heterologous for the recipient and often result in immune responses against the foreign proteins, such as β-gal, resulting in local inflammation and elimination of transduced cells (Brubaker et al., 1996; Juillard et al., 1995; Sarukhan et al., 2001; Yang et al., 1994; Yang et al., 1995).

The ROSA26 lacZ transgenic mice (Zambrowicz et al., 1997) (tolerant towards β-gal), has been used to analyze β-gal expression after lacZ gene transfer in some models (Vassalli et al., 1999; Yang, et al. et al., 1995) but high basal transgene-derived β-gal in some organs hampers its use.

The use of rats offers several advantages as compared to mice and in many cases rats represent an alternative and/or complementary model to mice. Due to its bigger size, the rat is more accessible than the mouse to microsurgery, multiple tissue and organ sampling, injection of substances into the brain followed by precise neuroanatomical localisation (in mice there is lack of resolution through diffusion seen in a smaller brain) and analysis of organ function ex vivo (eg. heart perfusion). Furthermore, a large body of physiological data and disease models (Gill et al., 1989) as well as transgenic technology (Charreau et al., 1999; Charreau et al., 1996) are available in the rat. Rats transgenic for reporter genes and with immune tolerance towards the transgene could represent a useful tool for the gene transfer field.

The aim of this study was to generate rats transgenic for lacZ with low β-gal expression, that would allow easy detection of lacZ gene transfer experiments, and displaying immune tolerance towards β-gal, which would eliminate immune responses against β-gal. Transgenic rats were obtained using a DNA construct in which lacZ was under the transcriptional control of the HIV-1 LTR, which has been shown to be expressed at low levels in lymphoid tissues.
and Langerhans cells from transgenic mice (Cavard et al., 1990; Leonard et al., 1989). These rats were analyzed for transgene-derived β-gal expression, for tolerance towards β-gal after immunisation with E. coli β-Gal or retrovirus-mediated gene transfer of lacZ, as well as for duration of retrovirus-mediated expression of β-gal.
MATERIALS AND METHODS

Generation of HIV-lacZ transgenic rats. Transgenic rats were produced following previously published procedures (Charreau, et al. et al., 1999; Charreau, et al. et al., 1996). Embryos were microinjected with a DNA construct (2 µg/ml), previously used to generate transgenic mice (Cavard, et al. et al., 1990) (kindly provided by P. Briand, Institut Cochin, Paris, France). This construct contained the U3R sequence of the HIV-1 LTR upstream of the cytoplasmic form of the lacZ gene and a SV40 polyadenylation signal. Tail DNA from the offspring was analyzed by PCR and Southern blot to identify transgenic founders carrying lacZ sequences.

Analysis of β–gal expression. For histochemical analysis, tissue biopsies were embedded in optimal cutting temperature compound (Miles Laboratories, Elkhardt, IN) and snap frozen in liquid nitrogen precooled methyl-butane. Cryostat sections (12 µm) were fixed and incubated with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal, Interchim, France) as previously described in detail (David et al., 1998). For immunohistological analysis, tissue biopsies were fixed in formalin and embedded in paraffin. Tissue sections (5 µm) were deparaffinized and incubated (18 h, 4°C) with a polyclonal mouse anti-β-gal antibody (Chemicon, Temecula, CA) diluted in PBS containing tween (0.1% v/v), then incubated with biotinylated goat anti-mouse immunoglobulin (Jackson Laboratories, West Grove, PA), horseradish peroxidase-conjugated streptavidin and amino ethyl carbazol substrate (Vector Laboratories, Burlingame, CA). The presence of β–gal was also analyzed in 200 µl of tissue homogenates (containing 250 µg of protein) using a sensitive (detection limit 40 pg/ml) ELISA kit according to the manufacturer’s (Boehringer Mannheim, Mannheim, Germany) instructions. For detection of β–gal transcripts, RNA was isolated and reverse transcribed as
previously described (David, et al., 1998). Primer sequences for β−gal were as follows: 5′-AGTTCAGATGTGCAGCGAGTT-3′ (sense) and 5′-TTCTCCAGCGACCAGAT-3′ (antisense). PCR was performed with 35 cycles of amplification at 94°C for 30 sec, 61°C for 30 sec and 72°C for 30 sec. The mRNA of hypoxanthine phosphoribosyltransferase was amplified as an internal control (David, et al. et al., 1998). Amplified sequences were blotted and hybridised with the respective probes labeled with 32P.

**Immunisations.** 100 µg of β−gal (Boehringer Mannheim) or KLH (Sigma, St. Louis, MO) emulsified in 200 µl of complete Freund's adjuvant (CFA) were injected into the footpad. Sera and draining lymph nodes were harvested ten days later.

**ELISA for detection of anti-β−gal and anti-KLH IgG antibodies.** Detection of anti-β−gal and anti-KLH IgG antibodies was performed by ELISA as previously described (Guillot et al., 2000), using recombinant β−gal (Sigma, l’isle d’Abeau, France). Results were expressed as the reciprocal serum dilution providing 50 % of the maximal optical density.

**Proliferation assays against-β−gal and anti-KLH.** Lymph node cells and splenocytes were cultured in 96-well plates (Nunc, Roskilde, Denmark) (10^5 cells/well) in triplicate cultures in RPMI culture medium (Guillot, et al. et al., 2000) in the presence of different concentrations of recombinant β−gal or KLH and concanavalin (ConA) (12.5 µg/ml) for 3 days. Proliferation was evaluated by 3H-thymidine incorporation during the final 8 hr of culture. For groups of animals, results were expressed as the mean proliferation index (proliferation against β−gal or KLH / proliferation in the presence of culture medium) ± SEM. For individual animals, results were expressed as the mean delta cpm (proliferation against β−gal or KLH – proliferation in the presence of culture medium) ± SD of triplicate wells.
**CTL assay against-β-gal-expressing cells.** Splenocytes were harvested > 30 days after retrovirus-mediated lacZ gene transfer and cultured for 5 days in the presence of recombinant vaccinia-lacZ viruses (kindly provided by Dr. Drillien, Strasbourg, France) at an M.O.I. of 10. Skin fibroblasts from newborn syngeneic rats either non-transduced or transduced with supernatants containing retroviruses coding for lacZ fused to a nuclear nls localisation signal (retro-nlslacZ) or GFP were used as target cells. Target fibroblasts were labeled with $^{51}$Cr and seeded in 96-well, V-bottom plates. Stimulated effector splenocytes were added to the target cells at various effector-to-target cell ratios. Spontaneous release (no effectors) and maximum release (10% SDS added) were included. The plates were incubated (6 h, 37°) and 50 µl of supernatant was removed and radioactivity determined. The percentage of specific $^{51}$Cr release was calculated as follows: (experimental release-spontaneous release) / (maximum release-spontaneous release) x 100. Spontaneous release was < 10%. Results were expressed as mean ± SD percentage of specific lysis for each group of animals at different target/effector ratios performed in triplicate.

**Retrovirus production and gene transfer in vivo.** Retro-nlslacZ were produced using the TELCeB6 AF7 cell line (Cosset *et al.*, 1995). Viral titers were $10^8$ transducing particles/ml. Two-thirds partial hepatectomy was performed as previously described (Ferry *et al.*, 1991) and retroviral delivery was performed 24 h after partial hepatectomy by i.v injection of 2.5 ml of medium containing retroviruses and 8 µg/ml of polybrene (Sigma).

**Statistics.** Statistical significance was evaluated using a one-way analysis of variance test and a p< 0.05 was considered as significative.
RESULTS AND DISCUSSION

Generation of rats transgenic for lacZ. The HIV-lacZ construct was microinjected into rat eggs and 146 of these embryos were transferred into foster mothers. Two rats among 33 pups were identified by Southern blot and PCR analysis as carrying the lacZ gene. Southern blot analysis of the progeny of both transgenic founders showed transgene transmission in both lines and the presence of 50 and 10 copies in lines 12 and 18, respectively (data not shown).

HIV-lacZ transgenic rats express ubiquitously low levels of β−gal.

In transgenic mice using the HIV LTR, transgene expression has been previously detected in eye tissues and hair follicles of embryos and in lymphoid tissues and skin Langerhans cells of adult mice (Cavard, et al. et al., 1990; Leonard, et al. et al., 1989). In rat embryos of both HIV-lacZ transgenic lines, β−gal expression was detected in eye tissues by X-gal staining (data not shown). In adult rats of both lines, analysis of β−gal mRNA levels showed low levels of expression in the thymus, lymph nodes, spleen, skin, liver, heart, lung and brain but none in the kidney, as detected by RT-PCR only after blotting and hybridization of the amplified sequences and not by northern blot analysis (Table 1). Analysis of β−gal expression by ELISA showed detectable levels of the protein in the thymus and spleen but not in the other organs of transgenic rats (Table 1). Analysis of β−gal expression by X-Gal and immunohistological staining of the lymphoid organs, skin, liver, heart, lung and kidney showed no labeling in any of these tissues (Table 1). Non-transgenic littermates used as negative controls in all experiments for analysis of β−gal expression at the mRNA or protein level did not display positive signals.

Thus, HIV-lacZ transgenic rats displayed undetectable levels of β−gal protein as assessed with the usual histology techniques used to detect β−gal after gene transfer. Low levels of
β-gal protein level were detected in the thymus and spleen suggesting that these animals could potentially display β-gal-specific immune tolerance by central and peripheral tolerance mechanisms.

HIV-lacZ transgenic rats display specific immune tolerance for β-gal. Antigen specific immune tolerance is defined as diminished or absent immune responses against a given antigen with normal immune responses against unrelated antigens being retained. To test whether HIV-lacZ transgenic rats displayed β-gal-specific immune tolerance, we immunized adult transgenic rats from line 12 or non-transgenic control animals via the footpad with β-gal or the unrelated KLH antigen using the strong complete Freund's adjuvant and analyzed their respective immune responses 10 days later. Lymphocytes of footpad draining lymph nodes from HIV-lacZ transgenic animals showed markedly lower proliferation against β-gal in vitro compared to those from immunized non-transgenic littermates but slightly higher than those of non-immunized controls (Fig. 1 A). In contrast, lymph node cells from HIV-lacZ transgenic and non-transgenic rats showed the same mean proliferation index using the non-specific mitogen ConA (58.8 ± 10.7, n=11 vs 46.4 ± 9.4, n=12, respectively). Addition of IL-2 increased proliferation of lymphocytes from HIV-lacZ transgenic lymph nodes, albeit at much lower levels compared to those of lymphocytes from non-transgenic rats (Fig. 1 A, insert). This result suggests that anergy, a peripheral tolerance mechanism, may play a role in tolerance of HIV-lacZ transgenic animals. Nevertheless, we can not exclude the possibility that tolerance is due to deletion in the thymus of high avidity anti-β-Gal T cells and that the remaining low-avidity T cells require IL-2 to proliferate in response to β-Gal.

After footpad injection, HIV-lacZ transgenic rats showed significantly reduced levels of anti-β-Gal IgG antibodies compared to those of non-transgenic littermates and higher levels than those of non-immunized controls (Fig. 1 B). Intraperitoneal immunisation with soluble β-Gal,
a weaker immunising protocol compared with the use of complete Freund’s adjuvant, also resulted in generation of anti-β-Gal antibodies by HIV-lacZ transgenic rats, albeit at much lower levels than in non-transgenic controls (data not shown). It can be concluded that HIV-lacZ transgenic animals displayed drastically reduced immune response against exogenous β−Gal, irrespectively of the immunisation protocol. To assess whether the reduction of anti-β-Gal immune responses in HIV-lacZ transgenic rats was due to Th1 to Th2 deviation, we analysed the isotype of IgG anti-β-Gal antibodies. In the rat, IgG2b predominantes in Th1 responses (Gracie and Bradley et al., 1996) whereas IgG1 and IgG2a predominate in Th2 responses (David, et al. et al., 1998). In HIV-lacZ transgenic rats immunised with β-Gal (n=10), IgG1, IgG2a and IgG2b anti-β-Gal antibodies showed a 2.7, 3.5 and 3.7-fold decrease, respectively, compared to the control group (n=12) (data not shown). Since Th1- and Th2-dependent IgG isotypes showed a comparable decrease, it can be concluded that Th1 to Th2 immune deviation does not play a role in tolerance towards β-Gal in HIV-lacZ transgenic rats.

When KLH was used as an immunogen, lymph node cells from HIV-lacZ transgenic and non-transgenic rats draining the footpad injected with KLH both showed comparably high proliferative responses whereas cells from non-immunized controls showed no proliferation (Fig. 1 C). Similarly, anti-KLH IgG antibodies were comparable in HIV-lacZ transgenic and non-transgenic rats immunized with KLH (Fig. 1 D). Injection of β−Gal into one footpad and of KLH in the contralateral footpad of the same transgenic (n=2) and non-transgenic (n=2) rats also revealed significantly decreased proliferative and antibody responses towards β−Gal and comparable immune responses to KLH in transgenic vs. non-transgenic animals (data not shown). Collectively, these results demonstrate specific tolerance towards β−gal in HIV-lacZ transgenic rats.
Persistent β–gal expression in HIV-lacZ transgenic rats after retrovirus-mediated gene transfer of lacZ. To explore the usefulness of HIV-lacZ transgenic rats in gene transfer protocols, we performed gene transfer of lacZ using retroviral vectors. Retroviruses can elicit primary immune responses but do not result in destruction of transduced cells due to absence of viral genes to be expressed by transduced cells. Retroviral-mediated gene transfer has been shown in certain cases to induce an immune response directed against the transgene product (Aubert et al., 2001; Izembart et al., 1999; Song et al., 1997). Gene transfer of lacZ into the liver of rats was performed using high doses of retro-nlslacZ. Delivery of retro-nlslacZ into non-transgenic rats resulted in approximately 10% of hepatocytes expressing β–gal-at days 7 and 14 but none at day 30 after gene transfer (Fig. 2 A, C and E). Livers of transgenic rats showed no detectable β–gal expression in the absence of gene transfer (Fig. 2 B, insert) and persistent expression at days 7, 30 and 260 after transduction with retro-nlslacZ (Fig. 2 B, D and F), suggesting that immune tolerance towards β–gal allowed a prolonged expression after gene transfer.

Absence of anti-β–gal immune responses in HIV-lacZ transgenic rats after retrovirus-mediated gene transfer of nlslacZ. To confirm that the persistent expression of β–gal in HIV-lacZ transgenic rats was associated with immune tolerance towards β–gal we analyzed the levels of antibodies, proliferation and CTL responses against β–gal in transgenic and non-transgenic rats after nlslacZ gene transfer.

After gene transfer of nlslacZ, anti-β–gal IgG antibodies were undetectable in HIV-lacZ transgenic rats, as for controls that did not receive retro-nlslacZ, whereas non-transgenic rats showed high titers of these antibodies (Fig. 3 A).

As for non-transduced controls, splenocytes from HIV-lacZ transgenic rats showed very low levels of proliferation but only in the presence of high concentrations of-β–gal with no
proliferation at lower levels (Fig. 3 B) In contrast, non-transgenic rats displayed higher proliferative responses at high and low concentrations of β−gal (Fig. 3 B). Proliferative responses to ConA were comparable in HIV-lacZ transgenic and non-transgenic rats (delta cpm for one animal of each group; 54,530 ± 811 vs 63,080 ± 215, respectively).

After gene transfer of nlslacZ, anti-β−gal cytotoxic T lymphocyte (CTL) activity was undetectable in splenocytes from HIV-lacZ transgenic rats, as for non-transduced controls, whereas significant CTL activity was detectable in splenocytes from non-transgenic rats (Fig. 3 C). Non-specific CTL activity against both non-transduced or GFP-transduced target cells was below 10% in both non-transgenic and transgenic splenocytes (data not shown).

Thus, indefinite β−gal expression by hepatocytes in HIV-lacZ transgenic rats is due to both tolerance towards β−gal and to the use of retroviruses as gene transfer vectors. Tolerance towards β−gal was included epitopes presented by MHC class II antigens, since anti-β−gal CD4+ cell proliferation and B cell responses were suppressed, and also by MHC class I antigens, since anti-β−gal CD8+ CTL responses were also absent. Anti-β−gal immune responses in non-transgenic rats may have resulted from either cross-priming of CD4+ T cells and CTLs by β−gal-derived peptides presented by MHC class II and I molecules on antigen presenting cells (APCs) or by direct transduction of APCs, as previously shown for retroviruses (Song, et al., 1997).

Importantly, anti-β-Gal immune responses were absent after lacZ gene transfer (Fig. 3) but only partially inhibited after injection of β-Gal (Fig. 1 A and B), as previously described in lacZ transgenic mice after injection with β-Gal (Salmon et al., 1998; Theopold and Köhler et al., 1990). This difference could be due to the use of an E. coli-derived non-glycosylated form of β-gal for immunization whereas the β-gal expressed by the cells after gene transfer is glycosylated. Alternatively, the presence of the β-gal protein in an extracellular form after footpad or systemic injection may be more immunogenic than intracellular β-gal expression.
after gene transfer of nlslacZ and therefore resulting in partial release of peripheral tolerance mechanisms. In confirmation of this idea, higher titers of anti-β-gal IgG antibodies were observed in non-transgenic animals injected with β-gal vs. those that received retro-lacZ (30,750 ± 5,221 vs. 4,983 ± 1,833, from Fig. 1 and 3 respectively). Increased immune responses have already been described after gene transfer of secreted or membrane vs. intracellular forms of β-gal due to more efficient antigen presentation (Sarukhan et al., 2001). β-gal offers several convenient features as a reporter gene. Precise cellular identification within tissues can be assessed in tissue sections by histochemical or immunohistochemical techniques. Furthermore, as is the case for GFP but not for other reporter genes, β-gal-expressing cells can easily be stained and sorted alive using fluorescein di-β-galactopyranoside (Nolan et al., 1988). There is minimal endogenous galactosidase activity whereas endogenous phosphatase alkaline activity (PAP) (MacGregor et al., 1995) and autofluorescence can interfere with the detection of PAP and GFP, respectively. Finally, in contrast to certain transgenic models overexpressing GFP (Huang et al., 2000; Martinez-Serrano et al., 2000), even very high levels of β-gal expression early in embryonic life has not been associated with toxicity (Zambrowicz, et al. et al., 1997). Rats transgenic for lacZ have been previously described (Charreau, et al. et al., 1996; Taniyama et al., 2001) but the use of promoters with non-lymphoid tissue-restricted specificity or even of immunoprivileged organs (such as the eye) and the lack of reported studies on immune tolerance towards the transgene product do not enable their use as tolerant recipients in gene transfer experiments. Rats transgenic for GFP (Hakamata et al., 2001) or PAP (Kisseberth et al., 1999) have been recently described and, although not reported, these animals could be tolerant for the transgenic proteins since transgene expression was high in immune cells. Nevertheless, these transgenes were expressed ubiquitously and at high levels making them unsuitable as recipients of gene transfer experiments due to high endogenous reporter gene expression.
In conclusion, HIV-lacZ transgenic rats displayed undetectable levels of β–gal as evaluated by histological techniques and were tolerant towards β–gal. These features enable their use as recipients in gene transfer and transplantation experiments using vectors or cells expressing β–gal as a reporter gene.
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REFERENCES


Table 1. Analysis of β–gal expression in organs from HIV-LTR transgenic in adult rats.

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<td>300 ± 100</td>
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<td>lymph nodes</td>
<td>+</td>
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Detection of β–gal mRNA was performed by RT-PCR followed by blotting and hybridisation with a radioactive probe (n=2-3). Detection of β–gal by ELISA (n=3) is expressed as pg of-β–gal/mg of total proteins. Immunohistology was performed on paraffin sections using an anti-β–gal antibody (n=2-3). Organs from non-transgenic rats were included in each experiment and were consistently negative for the three assays. Organs injected with an adenovirus coding for lacZ were used as positive controls in every immunohistology experiment.
**FIGURE LEGENDS**

**Fig. 1. Analysis of immune responses after immunization with β−gal or KLH.** HIV-lacZ transgenic rats and non-transgenic rats were immunized with 100 µg of either β−gal or KLH emulsified in CFA. Ten days later, draining lymph nodes and sera were harvested. **A)** Proliferation of lymph node cells from transgenic (squares, n= 11) non-transgenic (circles, n=12) or naïve (diamonds, n=4) animals against the indicated concentrations of β−gal. * p < 0.0002. Insert: experiments performed in the presence of IL-2 (50 U/ml). **B)** Anti-β−gal IgG antibodies were analyzed in serially diluted serum from transgenic (n=11), non-transgenic (n=12) and naïve (n=4) animals by ELISA. * p < 0.0002. **C)** Proliferation of lymph node cells draining the KLH injected footpad from transgenic (squares, n= 6) non-transgenic (circles, n=6) and naïve (diamonds, n=2) animals against the indicated concentrations of KLH. **D)** Anti-KLH IgG antibodies were detected in serially diluted serum from transgenic (n=6), non-transgenic (n=6) or naïve (n=2) animals by ELISA. Proliferation results are expressed as mean proliferation index ± SEM. ELISA results are expressed as the mean reciprocal serum dilution providing 50 % of the maximal optical density ± SEM.

**Fig. 2. Analysis of β−gal expression in hepatocytes of transgenic and non-transgenic rats after retrovirus-mediated gene transfer of lacZ.** HIV-lacZ transgenic rats and non-transgenic rats were partially hepatectomized, retro-mlslacZ was injected i.v 24 h later and liver were harvested at the indicated time points for analysis of β−gal expression using anti-β−gal antibodies. **(A, C and E)** Hepatocytes from non-transgenic rats display nuclear expression of β−gal at day 7 and 14 but not at day 30 after gene transfer. **(B, D and F)** Hepatocytes from HIV-lacZ transgenic rats display persistent nuclear expression of β−gal at day 7, 30 and 260 after gene transfer. The insert in D shows that β−gal positive hepatocytes in
HIV-lacZ transgenic rats, which did not receive retro-nlslacZ, were undetectable. Results are representative of 10 non-transgenic and 10 transgenic animals. Original magnification x 40.

Fig. 3. Analysis of anti-β−gal immune responses in transgenic and non-transgenic rats after retrovirus-mediated gene transfer of lacZ. HIV-lacZ transgenic rats and non-transgenic rats were partially hepatectomized, retro-nlslacZ was injected i.v. 24 h later and sera as well as spleens were harvested 21 days after gene transfer. (A) Anti-β−gal IgG antibodies were analyzed in the sera of non-transgenic (n=9), transgenic (n=8) or naïve (n=4) animals. * p=0.0002. (B) Splenocytes from transgenic (squares), non-transgenic (circles) or naïve (diamonds) rats were cultured for 3 days in the presence of β−gal at the indicated concentrations. Each curve corresponds to a single animal assayed in the same experiment and these results are representative of 5 other experiments with 11 transgenic and 10 non-transgenic animals. Results are expressed as mean proliferation index ± SD. (C) CTL activity of splenocytes from immunized transgenic (squares, n=4) non-transgenic (circles, n=4) or naïve (diamonds, n=2) rats was assayed against $^{51}$Cr labeled syngeneic fibroblasts expressing lacZ. * p < 0.003.
Fig. 1
Fig. 2
Fig. 3