Inflamed Glomeruli–Specific Gene Activation that Uses Recombinant Adenovirus with the Cre/loxP System

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Abstract. The authors previously reported that bone marrow–derived CD11b+CD18+ cells could be used as a vehicle to deliver foreign genes into inflamed glomeruli and that this vehicle cell (v-cell) could retard the progression of nephritis by delivering anti-inflammatory molecules. As a next step, the authors tried to establish a switching system by which v-cells are activated only at the inflamed glomeruli. A recombinant adenovirus (Ad) that expressed Cre recombinase under the control of the interleukin-1β (IL-1β) promoter (AxIL-1pr/Cre) was constructed and transfected into v-cells. After confirming that AxIL-1pr/Cre expresses Cre by lipopolysaccharide (LPS) treatment, AxIL-1pr/Cre was injected together with another Ad bearing a switching reporter unit in which the LacZ gene is activated under the control of the CAG promoter by the Cre-mediated excisional deletion of interposed stuffer DNA. Only a negligible number of double-infected (Cre/loxPCAG) cells expressed LacZ. This number, however, was significantly increased by LPS, which suggests that LPS-induced Cre effectively deletes the stuffer DNA, which allows for a complete CAG promoter. DBA/2j mice were then transplanted with Cre/loxPCAG cells via a tail vein and treated with anti–glomerular basement membrane (GBM) serum. To trace the transplanted cells, marker v-cells, infected with AxCANLacZ to constitutively express the LacZ gene, were also used. Although transplanted cells expressing LacZ collected in the spleen independent of anti-GBM treatment, they did not express the LacZ gene in the mice transplanted with Cre/loxPCAG cells. On the other hand, transplanted cells were recruited in the glomeruli and expressed the LacZ gene upon anti-GBM treatment. These results suggested that only the v-cells recruited in the glomeruli could be switched on and activate foreign genes.

Glomerulonephritis, in most cases, progresses toward end-stage damage, despite active treatment with drugs such as steroid hormones, and throughout the world a large number of patients have had to undergo dialysis. This suggests the need for a next-generation therapy against glomerulonephritis that can reduce the problems that are encountered in conventional therapy. We previously used bone marrow cells to establish an inflamed site-specific gene delivery system (1). Before transplantation, bone marrow cells were differentiated into monocyte-lineage cells ex vivo to express CD11b and CD18, both of which are ligands of intercellular adhesion molecule–1 (2), so that these cells could be recruited at the site of intercellular adhesion molecule–1 expression. Using this system, we retarded glomerular inflammation during the development of glomerulonephritis by delivering an anti-inflammatory cytokine (3). However, these genetically modified cells were also collected in other tissues, such as spleen, without any association with adhesion molecules (3). Therefore, as the next step toward clinical application, we tried to establish a switching system by which vehicle cells are activated only at the inflamed site.

The proinflammatory cytokine interleukin (IL)-1β plays an important role in the initiation and progression of glomerulonephritis (4,5). During the progression of glomerulonephritis, macrophages that have migrated into the inflamed glomeruli secrete IL-1β, which initiates and/or enhances the process of glomerular inflammation (6). This suggests that glomerular inflammation alters the microenvironment to switch on the IL-1β promoter in the migrated cells. We therefore focused on the IL-1β promoter as a marker of glomerular inflammation so that inflamed site-specific activation might be achieved. The use of this promoter may, however, be limited because the expression level is low (7) and may not be sufficient for effective gene expression to prevent progression of glomerulonephritis. We previously used chicken β-actin promoter to express IL-1 receptor antagonist (IL-1Ra). Using this promoter, we successfully expressed enough IL-1Ra to cancel the IL-1β action and suppress renal injury during anti-GBM glomerulonephritis. Therefore, a gene activation system that is regulated by the IL-1β promoter but driven by the stronger promoter should be developed.
Cre recombinase derived from bacteriophage P1 is a 38-kD protein that mediates excisional deletion of a DNA sequence flanked by a pair of loxP sites, which is composed of two 13-bp inverted repeats separated by an 8-bp spacer region (8). When it binds to the inverted repeats, Cre synapses with a second loxP site and then cleaves the DNA in the spacer region to initiate strand exchange with the synapsed loxP partner. The gene-activation strategy involves the excisional deletion of “stuffer DNA” that lies between the promoter and the coding region and prevents expression. Cre is an enzyme; therefore, a small amount of expressed Cre may process large amount of molecules. Even though a weak promoter regulates the transfected gene, the Cre/loxP system may change it into a stronger promoter, which allows it to enhance the gene expression. Sato et al. (9) recently reported that the Cre/loxP system may give about 50-fold higher expression than the single transfection directly driven by the original promoter. Therefore, we constructed a “regulator” adenovirus that bears Cre under the control of the IL-1β promoter and double-infected vehicle cells with the “target” adenovirus containing a Cre-activating reporter unit driven by the strong CAG (cytomegalovirus enhancer-chicken β-actin hybrid) promoter (10) and confirmed the regulation in vitro and in vivo. In this study, we combined our gene delivery system with the Cre/loxP system for site-specific gene activation to reduce the unexpected side effects of delivering multipotent anti-inflammatory cytokine to other tissues.

Materials and Methods

Experimental Design

We constructed a regulator recombinant adenovirus that produces Cre under the control of the IL-1β promoter (AxIL-1pr/Cre) (Figure 1). After confirming that AxIL-1pr/Cre introduces the Cre gene into vehicle cells and that its expression is enhanced by lipopolysaccharide (LPS) treatment using PCR and Western blot analysis, respectively, we transfected AxIL-1pr/Cre together with a target adenovirus, AxCALNLNZ (Figure 1), which bears a switching reporter unit in which a LacZ gene can be activated under the control of the CAG promoter (AxCANLacZ) (Figure 1), expressing the LacZ gene with an intact CAG promoter were used. Kidney and spleen tissue sections infected with AxCANLacZ (Figure 1) expressing the LacZ gene with adjuvant and rabbit IgG 4 d earlier and then treated with anti-LPS. These cells were then infused into DBA/2j mice preimmunized with adjuvant and rabbit IgG 4 d earlier and then treated with anti-GBM serum 24 h later. To trace the transplanted vehicle cells, cells infected with AxCANLacZ (Figure 1) expressing the LacZ gene with an intact CAG promoter were used. Kidney and spleen tissue sections from each group of mice were subjected to X-gal assay to examine the activation of the reporter gene in vivo.

Animals

Seven-week-old female DBA/2j mice were purchased from Nippon Crea (Tokyo, Japan). All animals used in this study were maintained in our animal facility on standard laboratory chow.

Establishment of Vehicle Cells

CD11b and CD18 positive vehicle cells were established as described elsewhere (1). In brief, bone marrow cells were harvested from the femur, tibia, and pelvis of 7- to 8-wk-old mice and suspended in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 20% heat-inactivated horse serum, 20% L-929 conditioned medium (11), 100 U/ml penicillin G, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. Cells were seeded onto unprocessed 10-cm dishes at a concentration of 1 × 10^7 cells per dish and cultured in a humidified atmosphere of 5% CO_2 for 1 wk. The population of CD11b and CD18 positive cell was verified to be homologous by flow cytometry (data not shown).

Preparation and Infection of Recombinant Adenovirus

The replication-defective recombinant adenovirus, AxIL-1pr/Cre, was constructed essentially according to reports elsewhere (12,13). To generate AxIL-1pr/Cre, we first cloned IL-1β promoter cDNA (kindly donated by Dr. C.J. Ballone, St. Louis University [14]) into a cassette cosmid pAxAwNCre (a gift from Dr. I. Saito, University of Tokyo) that carried an adenovirus type-5 genome lacking the E3, E1A, and E1B regions to prevent replication. In this construct, Cre cDNA is located downstream of the IL-1β promoter and is followed by the rabbit β-globin poly(A) sequence. The resulting cosmid was cotransfected to 293 cells with the appropriately cleaved adenovirus genome lacking the E3 region. Recombinant virus was propagated and isolated from 293 host cells. AxCANCre, AxCANLacZ, and AxCALNLNZ were purchased from RIKEN cell bank (Ibaraki, Japan). Bone marrow cells were cultivated with Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 20% heat-inactivated horse serum, and 20% L929-conditioned medium for 6 d and then infected

Figure 1. Structures of adenoviruses. AxIL-1pr/Cre expresses Cre recombinase under the control of the IL-1β promoter. AxCANCre and AxCANLacZ express the Cre recombinase and LacZ gene, respectively, under the control of the CAG promoter. AxCALNLNZ is designed to express the LacZ gene only after Cre-mediated excisional deletion of stuffer DNA. Solid lines indicate the recombinant adenovirus genome, and arrows show the orientation of the transcription. IL-1pr, IL-1β promoter; pA, rabbit β-globin poly(A) site; SpA, SV40 early poly(A) site.
min at 50 °C. The samples were then extracted with phenol-chloro-
agents (Amersham, Buckinghanshire, UK).

antibody complexes were visualized with chemiluminescence re-
with goat anti-mouse Ig horseradish peroxidase conjugate. Antigen-
monoclonal antibody (BabCO, Richmond, CA). It was then incubated

Tween 20, and PBS and incubated with an anti-Cre recombinase

membrane. The membrane was blocked with 5% dried milk, 0.1%
polyacrylamide gel and then transferred to nylon
dodecyl sulfate
grams of protein were subjected to electrophoresis in a 12.5% sodium

PCR for Cre Recombinase

PCR for Cre recombinase was performed as described elsewhere (15). Briefly, vehicle cells with or without transfection were har-
estimated, suspended in a lysis buffer (50 mM Tris-HCl [pH 7.5], 100
mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium
vanadate, 1 mM DTT, 10 mmol/L sodium fluoride, 10 μg/ml each of leupeptin and aprotinin, and 1% Brij-96). Two micro-
grams of protein were subjected to electrophoresis in a 12.5% sodium
dodecyl sulfate–polyacrylamide gel and then transferred to nylon
membrane. The membrane was blocked with 5% dried milk, 0.1%
Tween 20, and PBS and incubated with an anti-Cre recombinase
monoclonal antibody (BabCO, Richmond, CA). It was then incubated
with goat anti-mouse Ig horseradish peroxidase conjugate. Antigen-
antibody complexes were visualized with chemiluminescence re-
agents (Amersham, Buckinghamshire, UK).

92°C for 80 s; annealing, 65°C for 60 s; and elongation, 72°C for
90 s). After electrophoresis in a 2% agarose gel, amplified products
were visualized with ethidium bromide staining.

Induction of Anti-GBM Glomerulonephritis

Glomerulonephritis was induced with an anti-GBM nephrotoxic
serum as described elsewhere (16). In brief, GBM was prepared from
Wister Kyoto rats (Charles River Japan, Inc., Kanagawa, Japan) by a
differential sieving technique and then by sonication and centrifuga-
rabbit anti-GBM nephrotoxic serum was raised by repeated
immunization of a Japanese White rabbit (Kitayama Labes Co., Ltd.,
Nagano, Japan) with particulate GBM. The anti-GBM serum was
pooled and decomponented at 56°C for 30 min. To induce anti-GBM
glomerulonephritis, DBA/2j mice were intraperitoneally injected with
0.5 mg/20 g body wt of normal rabbit IgG (Cappel, West Chester, PA)
and complete Freund’s adjuvant (1:1 dilution; Difco, Detroit, MI).
The crossreactivity of this serum with murine GBM was certified by
immunohistochemistry, which detected the rabbit IgG on the glomer-
uli 3 d after injection (data not shown).

Western Blot Analysis

Western blot analysis was performed on the Cre secreted from
transfected vehicle cells as described elsewhere (3). Briefly, after transfection with AxIL-1pr/Cr and AxCALNLNZ, vehicle cells were
cultured for 48 h with or without LPS in a 6-well plate. Cell were
harvested and suspended in lysis buffer (50 mmol/L Tris [pH 7.6], 150
mmol/L NaCl, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L
sodium vanadate, 1 mmol/L DTT, 10 mmol/L sodium fluoride, 10
μg/ml each of leupeptin and aprotinin, and 1% Brij-96). Two micro-
grams of protein were subjected to electrophoresis on a 12.5% sodium
dodecyl sulfate–polyacrylamide gel and then transferred to a nylon
membrane. The membrane was blocked with 5% dried milk, 0.1%
Tween 20, and PBS and incubated with an anti-Cre recombinase
monoclonal antibody (BabCO, Richmond, CA). It was then incubated
with goat anti-mouse Ig horseradish peroxidase conjugate. Antigen-
antibody complexes were visualized with chemiluminescence re-
agents (Amersham, Buckinghamshire, UK).

X-gal Assay

X-gal assay was performed to assess the expression of the LacZ
gene as described elsewhere (17) with a small modification. In brief,
cells or tissue cryosections (6 μm) were fixed with 0.25% glutaral-
dehyde in PBS for 10 min at 4°C and incubated at 37°C for 3 h in
a reaction buffer that contained 1 mg/ml X-gal (4-Cl-5-Br-3-indolyl-β-
galactosidase), 5 mM potassium ferricyanide, 5 mM potassium ferro-
cyante, and 2 mM MgCl2 in PBS. LacZ-positive cells were stained
in blue. To quantify the recruited vehicle cells in the glomerulus, >50
glomeruli per mouse were randomly assessed, and glomeruli contain-
ing LacZ positive cell(s) were counted. Values were expressed as the
mean ± SEM (%).

Results

Transfection into Vehicle Cells and Regulation of
AxIL-1pr/Cr

A recombinant adenovirus that expressed Cre recombinase
under the control of the IL-1β promoter (AxIL-1pr/Cr) was
constructed and infected into primarily established vehicle
cells. DNA was extracted from AxIL-1pr/Cr-transfected
vehicle cells and subjected to PCR specific for Cre recombinase
cDNA. As shown in Figure 3, the 269-bp band, which is
specific for the bacteriophage Cre recombinase gene (15), was
seen in infected cells. To confirm that the IL-1β promoter
regulates the expression of Cre recombinase, AxIL-1pr/Cr-
transfected vehicle cells were treated with or without LPS (3

Figure 2. Inflamed site-specific gene activation using the Cre/loxP
system. Cre recombinase expressed by the IL-1β promoter excises the
stuffer DNA from the AxCALNLNZ genome and consequently gen-
erates LacZ expression driven by a potent CAG promoter.

Figure 3. Adenoviral transfection of Cre recombinase gene under
the control of the IL-1β promoter into vehicle cells. Freshly established
vehicle cells were transfected with IL-1pr/Cr and subjected to PCR
for the Cre recombinase gene. The 269 bp gene product corresponds
to bacteriophage Cre recombinase.
Regulation of Gene Expression by the Cre/loxP System

To elucidate whether the reporter gene is activated by the Cre/loxP system in vitro, AdxIL-1pr/Cre was cotransfected with the “target” adenovirus AxCALNLNZ bearing a switching reporter unit, whose LacZ gene is activated under the control of the CAG promoter by the Cre-mediated excisional deletion of interposed stuffer DNA. As a control, AxCACre expressing Cre recombinase under the control of the intact CAG promoter was also cotransfected with AxCALNLNZ into vehicle cells. As shown in Figure 5, only a negligible number of AxIL-1pr/Cre and AxCALNLNZ double-transfected cells expressed the LacZ gene, whereas these cell numbers were significantly increased by LPS treatment. On the other hand, the cells that were double-transfected with AxCACre and AxCALNLNZ, in which Cre recombinase was constitutively induced, expressed the LacZ gene independent of LPS treatment. These results indicate that LPS-induced Cre recombinase effectively deletes the stuffer DNA, which results in a complete CAG promoter, which induces the LacZ gene.

Regulation of Gene Expression by the Cre/loxP System

To examine whether the transgene is activated by the Cre/loxP system in vivo, DBA/2j mice preimmunized with adjuvant and rabbit IgG were injected with vehicle cells, double-transfected with AxIL-1pr/Cre and AxCALNLNZ via a tail vein, and treated with anti-GBM serum 24 h later. Three days later, mice were killed and tissue specimens were subjected to X-gal staining to assess the LacZ expression. To trace the transplanted vehicle cells, AxCANLacZ-infected marker cells, which constitutively express the LacZ gene under the control of the CAG promoter (Figure 1), were infused to mice by following the same protocol. In the mice transplanted with marker cells, LacZ-positive cells were detected in the spleen regardless of anti-GBM treatment (Figure 6, a and b). Only a negligible number of LacZ-positive cells were detected in the kidney (0.4 ± 0.1% of glomeruli contained LacZ-positive cell; Figure 6c), whereas many cells were detected in the glomerulus 3 d after anti-GBM treatment (85.6 ± 7.7% of glomeruli contained LacZ-positive cells; Figure 6d). Because β-galactosidase was widely exudated, it is difficult to specify the location of LacZ-positive cells; however, it seems to have been in the mesangial area (Figure 6, e through g and Figure 7e through g). Using two-color immunofluorescence staining with anti-β galactosidase and F4/80, we confirmed that the LacZ-positive cells recruited in glomeruli still possessed the property of monocyte-lineage cells (data not shown). These results suggest that transplanted cells accumulated in spleen independent of inflammation and were recruited to the inflamed glomeruli upon treatment with anti-GBM serum. On the other hand, transplanted cells must be accumulated in the spleen; however, almost no LacZ-positive cells could be detected in spleen from the mice transplanted with double-transfected cells. In contrast, LacZ-positive cells were detected in the glomeruli of these mice (Figure 7d). The kinetics of the number of LacZ-positive glomeruli is shown in Figure 7h. It peaked at day 3 (79.4 ± 10.9% of glomeruli contained LacZ-positive cells) and decreased later, which corresponds to our previous data that CD11b+CD18+ (Mac-1+) cells were detected in glomeruli 3 d after anti-GBM serum injection but translocated later into interstitium until 7 d (our unpublished data). Because there are no significant pathologic changes at this time point (data not shown), we could not prove the relevance of the severity of glomerular damage and the number of infiltrated LacZ-positive cells. However, these results suggest that the vehicle cells that collected in the spleen were not activated, whereas the cells recruited to the inflamed glomeruli could be switched on to activate the LacZ gene.

Figure 4. Regulation of Cre recombinase expression by the IL-1β promoter in the IL-1pr/Cre transfected vehicle cells. Freshly established vehicle cells were transfected with IL-1pr/Cre and treated with or without lipopolysaccharide (LPS), which activates the IL-1β promoter in vehicle cells. These cells were subjected to Western blot analysis of the expression of Cre recombinase. The 38-kD protein corresponds to Cre recombinase.
Discussion

The Cre/loxP system has recently been used for gene activation and inactivation in transgenic mice (19,20) as well as for activation of a transgene located in the adenovirus genome (21) and on a cell chromosome (22). In this study, we applied this gene activation strategy to the development of an “inflamed site-molecular switch” by using an adenovirus that expresses Cre recombinase under the control of the IL-1β promoter. The Cre-producing virus was used to activate the reporter gene under the control of a potent CAG promoter in the second adenovirus genome. Double transfection of these two adenoviruses achieved inflamed site-specific activation, which was maintained in vitro and in vivo.

It was reported that in vivo gene activation within the glomerulus could be achieved by use of a tetracycline regulatory system (23) by which expression of the reporter gene in the cloned mesangial cell trapped in the glomerular vasculature could be regulated by oral tetracycline administration (24). This approach may be described as manual regulation; however, during the development of chronic inflammation, the activity for local inflammation is turned on and off and is difficult to assess by systemic manifestation for the period of drug administration. Therefore, an automatic regulatory system by which gene expression is controlled depending on the activity for inflammation would be ideal. In this regard, Kitamura and Kawachi (25) used the promoter of α-smooth muscle actin to induce a transgene, because the expression of this actin is markedly up-regulated in mesangial cells in a wide range of experimental and human glomerular diseases (26,27), and established an automatic on/off switching system to express the transgene only where glomerular inflammation occurs and deactivate it when the inflammation has subsided (25). Such success may strengthen the rationale for therapeutic gene delivery for the treatment of glomerular inflammation. The use of this system, however, is limited because gene activation by the α-smooth muscle actin promoter is restricted in glomerular mesangial cells. Also, because stable gene transfection into residential mesangial cells in vivo has yet to be accomplished, it is difficult to apply this system directly to therapeutic intervention, at least in its current form. Our gene delivery system

Figure 6. Location of vehicle cells after transplantation in mice treated with or without anti-GBM serum. Vehicle cells were labeled with AxCANLacZ to constitutively express the LacZ gene and infused to mice. These mice were treated with or without anti-GBM serum and killed 3 d later. Spleen and kidney specimens were subjected to X-gal assay. Five mice per group were examined, and representative pictures are shown. (a) Spleen of the mouse without anti-GBM serum. (b) Spleen of the mouse with anti-GBM serum. (c) Kidney of the mouse without anti-GBM serum. (d through g) Kidney of the mouse with anti-GBM serum. Magnifications: ×100 in a through d; ×400 in e through g.
is based on the genetic manipulation of bone marrow–derived cells, which are recruited at the site of inflammation in association with adhesion molecule(s). Therefore, we sought another promoter for automatic gene activation, which restricted the recruited inflammatory cells and focused on the IL-1β promoter, because macrophages recruited to the inflamed glomeruli secrete IL-1β, which initiates and/or enhances the progression of glomerular inflammation, suggesting that glomerular inflammation alters the microenvironment to switch on the IL-1β promoter in the migrated cells. Furthermore, because the

Figure 7. Cre/loxP system–mediated gene expression in vivo. Vehicle cells were double-transfected with AxIL-1pr/Cre and AxCALNLNZ and infused to mice. These mice were treated with or without anti-GBM serum and killed at day 0, 1, 3, 5, 7. Spleen and kidney specimens were subjected to X-gal assay. Representative pictures of day 3 are shown. (a) Spleen of the double-transfected mouse without anti-GBM serum. (b) Spleen of the mouse with anti-GBM serum. (c) Kidney of the mouse without anti-GBM serum. (d through g) Kidney of the mouse with anti-GBM serum. (h) Quantitative analysis. Percentages of glomeruli containing the LacZ-expressing cells were determined and described as % LacZ positive glomeruli (%). Magnifications: ×100 in a through d; ×400 in e through g.
IL-1β gene is inactivated in most resting mononuclear phagocytes and a stimulus was required to induce its expression (28–30), basal activation of the IL-1β promoter without a stimulus may be quite low, as is suggested by Figures 3 and 4. One major problem with the use of this promoter was the low-level induction after stimulation (7), which may not be sufficient for gene delivery to cancel the inflammatory cytokine(s). Our previous study confirmed that the chicken β-actin promoter induces enough IL-1Ra to cancel IL-1β action during the development of glomerulonephritis (3). Therefore, a gene activation system, which is regulated by the IL-1β promoter but driven by the CAG promoter, was established by combining the IL-1β promoter and Cre/loxP system. In this study, we showed that regulation is achieved in the vehicle cells in vitro and in vivo.

This system, however, has several disadvantages compared with the previous gene activation systems. First, the ideal system should be switched on at the initiation of inflammation and switched off at its cessation. The Cre/loxP system combined with the IL-1β promoter is a one-way activation system and could not switch off the gene expression even after the IL-1β promoter was deactivated. In this regard, Kruth et al. (31) reported that transplanted macrophages disappear within 4 d of their recruitment to inflamed glomeruli. Furthermore, we used an adenovirus to introduce both a regulator and a target gene, because other conventional methods that have used liposome, calcium phosphate coprecipitation, and retrovirus could not effectively transduce foreign genes into vehicle cells (unpublished data). Gene expression, therefore, may be high but transient. We believe that such natural elimination may overcome the disadvantage of this system. Second, as shown in Figure 7, only a small number of vehicle cells could be recruited into the glomeruli even after anti-GBM serum administration when this gene delivery system was used. In this regard, we previously reported that delivery of IL-1Ra, which is driven by chicken β-actin promoter, may suppress the glomerular inflammation in the anti-GBM glomerulonephritis even if only a few vehicle cells may be recruited by this insult (3), which suggests that therapeutic efficacy does not depend on the number of recruited cells but on the amount of secreted transgene. Because adenovirus may transduce multiple copies of the foreign gene and because those may be driven by the strong promoter, the anti-inflammatory molecules secreted from a small number of vehicle cells may cancel the proinflammatory cytokines from native cells. This fact strengthens the rationale of using adenovirus-mediated Cre/loxP system with our gene delivery system because this may provide the strong gene activation while maintaining inflamed-site specificity.

In conclusion, we succeeded in that inflamed glomeruli-specific gene activation was achieved when the Cre/loxP system was combined with the IL-1β promoter. We recently modified this system and established “vehicle cell–producing tissue” by repetitive injection of retrovirally modified hematopoietic stem cells into sublethally irradiated mice to prolong the restricted time window of the current system (32). We have only just begun this research, and many problems must be solved before any major investment in clinical use can be made. We still believe that these trials represent the next step in research aimed at the discovery of a novel therapeutic strategy for glomerulonephritis.

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References

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