Decoy receptor 3 inhibits renal mononuclear leukocyte infiltration and apoptosis and prevents progression of IgA nephropathy in mice

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Ka S, Hsieh T, Lin S, Yang S, Wu C, Sytwu H, Chen A. Decoy receptor 3 inhibits renal mononuclear leukocyte infiltration and apoptosis and prevents progression of IgA nephropathy in mice. Am J Physiol Renal Physiol 301: F1218–F1230, 2011.—The progression of IgA nephropathy (IgAN), the monotypic type of primary glomerulonephritis, is associated with high levels of mononuclear leukocyte infiltration into the kidney. These cells consist mainly of T cells and macrophages. Our previous study showed that a decoy receptor 3 (DCR3) gene therapy can prevent the development of a mouse autoimmune glomerulonephritis model by its potent immune modulating effects (Ka SM, Sytwu HK, Chang DM, Hsieh SL, Tsai PY, Chen A. J Am Soc Nephrol 18: 2473–2485, 2007). Here, we tested the hypothesis that DCR3 might prevent the progression of IgAN, an immune complex-mediated primary glomerulonephritis, by inhibiting T cell activation, renal T cell/macrophage infiltration, and protecting the kidney from apoptosis. We used a progressive IgAN (Prg-IgAN) model in B cell-deficient mice, because the mice are characterized by a dramatic proliferation of activated T cells systemically and progressive NF-κB activation in the kidney. We treated the animals with short-term gene therapy with DCR3 plasmids by hydrodynamics-based gene delivery. When the mice were euthanized on day 21, we found that, compared with empty vector-treated (disease control) Prg-IgAN mice, DCR3 gene therapy resulted in 1) systemic inhibition of T cell activation and proliferation; 2) lower serum levels of proinflammatory cytokines; 3) improved proteinuria, renal function, and inflammation (inhibiting the development of marked glomerular proliferation, crescent formation, glomerulosclerosis, and interstitial inflammation); 5) suppression of T cell and macrophage infiltration into the periglomerular interstitium of the kidney; and 5) a reduction in apoptotic figures in the kidney. On the basis of these findings, DCR3 might be useful therapeutically in preventing the progression of IgAN.

hydrodynamics-based gene delivery; T cell; macrophage

PROGRESSION DURING THE COURSE of IgA nephropathy (IgAN) is considered to be a key step in the subsequent development of end-stage renal disease (3, 61), but it is relatively unpredictable and clinically remains a challenge in terms of prophylaxis and treatment. Although certain clinical, immunological, molecular, and pathological parameters are considered to contribute to the progression of IgAN patients (35, 48, 53), T cells play a major role in the unfavorable clinical situation (6, 46, 47). Besides, in most types of glomerulonephritis, progression to chronic renal failure is closely correlated with high levels of mononuclear leukocytic infiltration into the kidney (1, 27, 31). The mononuclear leukocytes are often found around inflamed glomeruli (periglomerular infiltration) and consist mainly of T cells (14, 58), macrophages (55, 59), and dendritic cells (15). Decoy receptor 3 (DCR3), a potential immune regulator, can directly modulate the biological function of T cells (22, 24, 49, 66), macrophages (4), or dendritic cells (21, 63). Importantly, DCR3 enhances the survival and metastasis of cancer cells by modulating immune responses (5, 19), and it promotes the growth of tumor cells by helping them avoid an immune attack as a result of lymphocyte infiltration and FasL/LIGHT-mediated apoptosis (44, 64). It is likely that the inhibitory role of DCR3 on immune responses, especially the modulation of the activity of mononuclear leukocytes, can be renoprotective against immunologically enhanced exacerbation and deterioration of IgAN. Our previous study showed that gene therapy with DCR3 plasmids can prevent the development of an autoimmune crescentic glomerulonephritis model in mice by inhibiting systemic T cell activation/proliferation and B cell activation, apoptosis, and intrarenal mononuclear leukocyte infiltration (24) and that transgenic DCR3 protects mice from autoimmune- and cyclophosphamide-induced diabetes in a dose-dependent manner and significantly reduces the severity of insulin in an autoimmune diabetes model (52). In agreement with these findings, Zhang et al. (66) reported that an in vivo administration of DCR3 ameliorates allograft rejection. All these studies provide evidence that DCR3 is an immunomodulator suitable for development as a therapeutic agent for controlling undesirable immune responses. However, for primary glomerulonephritis such as IgAN, such an effect of DCR3 has yet to be determined.

We established a progressive IgAN (Prg-IgAN) model in B cell-deficient (BCD) mice that is suitable for the investigation of progression of IgAN (6). The features of the model are 1) greatly enhanced glomerular proliferation, including focally crescentic formation; 2) periglomerular mononuclear leukocytic infiltration; and 3) a rapid decline in renal function, all associated with a dramatic increase in the number of activated T cells (CD3+ CD69+) and progressive activation of nuclear NF-κB transcription factor. All these pathological features are often identified in Prg-IgAN patients (2, 12).

In the present study, we tested the hypothesis that short-term DCR3 gene therapy might prevent the progression of IgAN in BCD mice by inhibiting systemic T cell activation and proliferation, renal T cell/macrophage infiltration, and protecting the kidney against apoptosis.
MATERIALS AND METHODS

Establishment of Prg-IgAN Mouse Model

BCD mice (B6.129S2-Igh-6tm1Cgn/J) were obtained from Professor John T. Kung, (Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan) and maintained at the animal center of the National Defense Medical Center (Taipei, Taiwan). Prg-IgAN was induced in BCD mice by a daily injection of purified IgA anti-phosphorycholine antibodies and pneumococcal C-polysaccharide (PnC), as described previously (6). All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of The National Defense Medical Center, Taiwan, and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

DCR3 Gene Therapy

Plasmid human DCR3 cDNA (sequence data available from GenBank/EMBL/DDBJ under accession no. AF104419) was produced as described previously (24). Briefly, the DCR3 gene was isolated by RT-PCR using forward primer 5-CAAGGACCATGAGGGCGCTG-3 and reverse primer 5-GTGCACAGGGAGGAAGCGC-3 and was subcloned into the pCMV vector (Clontech, Palo Alto, CA) to produce the pCMV-DCR3 expression construct.

The expression construct or the empty vector was administered to the mice by hydrodynamics-based gene delivery using the dose intervals of treatment described previously (24, 34) with minor modifications. We injected a single dose of DCR3 plasmid into normal BCD mice that received DCR3 only were used as normal controls. IgAN mice given the empty vector were used as disease controls, and normal BCD mice that received DCR3 only were used as normal controls.

Clinical and Pathological Evaluation

Collection and assay of blood and urine samples were performed as described previously (24). Urine samples were collected in metabolic cages weekly, and urinary levels of protein were determined using a Pierce BCA protein assay kit (Perbio Science, Etten-Leur, The Netherlands), while serum samples were collected at days 3, 14, and 21 to measure blood urea nitrogen (BUN) and serum creatinine (Cr).

For histopathology, the tissues were fixed in 10% buffered formalin and embedded in paraffin, and then sections (4 μm) were prepared and stained with hematoxylin and eosin (H&E). One hundred glomeruli were examined in at least two renal tissue sections per slide by light microscopy at a magnification of ×400. The severity of renal lesions was scored as described previously (24), and the percentage of glomeruli showing proliferation, glomerular crescent, glomerular sclerosis, or periglomerular inflammation was calculated.

Real-Time PCR Assay

RNA extracted from the kidney cortex with TRizol reagents (Invitrogen, Carlsbad, CA). For first-strand cDNA synthesis, 1.5 μg of total RNA was used in a single-round RT reaction. The reaction mixture consisted of 0.9 μl of oligo (dT) 12 to 18 primer, 1.0 mM deoxyribonucleotide triphosphate (dTTP), 1 μl first-strand buffer, 0.4 mM dithiothreitol, 80 U of RNaseout recombinant RNase inhibitor, and 300 U of superscript II RNase H (Invitrogen). Real-time PCR was performed with the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). All of the probes and primers were Assays-on-Demand Gene expression products (Applied Biosystems). Real-time PCR reactions were done using 10 μl of cDNA, 12.5 μl of TaqMan Universal PCR Master Mix (Applied Biosystems), and 1.25 μl of the specific probe/primer mixed in a total volume of 25 μl. The thermal cycler conditions were as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles of denaturation (15 s at 95°C), and combined annealing/extension (1 min at 60°C). The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase was used as the internal standard.

Immunofluorescence, Immunohistochemistry, and Detection of Apoptosis

For the detection of bound IgA and C3 by immunofluorescence, frozen renal tissues were cut, air-dried, fixed in acetic for 5 min at room temperature, and incubated with FITC-conjugated goat anti-

Fig. 1. Hepatic expression of decoy receptor 3 (DCR3). A: real-time PCR for DCR3 mRNA. B: Western blot analysis for DCR3 protein. The arrow indicates the time of gene delivery with DCR3 plasmid or empty vector plasmid. Values are means ± SE for groups of 12 mice. ***P < 0.005 compared with day 0.
mouse IgA or C3 antibodies (Cappel, Durham, NC). Staining intensity was scored as described previously (6, 24).

For immunohistochemistry, methyl Carnoy’s solution-fixed or formalin-fixed, paraffin-embedded tissue sections (4 μm) were stained with goat anti-collagen IV (Col-IV) antibodies (SouthernBiotech, Birmingham, AL), rabbit anti-fibronectin (FN) antibodies (Chemicon, Temecula, CA), rabbit anti-phosphorylated NF-κB p65 (Cell Signaling, Beverly, MA), or rat anti-F4/80+ antibodies (macrophage marker; Serotec, Raleigh, NC), or frozen sections of renal tissues fixed in periodate-lysine paraformaldehyde as described previously (24) were incubated with goat anti-MCP-1, rabbit anti-IL-6 (Santa Cruz Biotechnology, Santa Cruz, CA), biotin-conjugated antibodies against mouse CD3 (Serotec), or CD11b (BD Biosciences, San Jose, CA). The intensity of staining of the glomerulus (including the periglomerular area) was scored as described previously (6, 24). For detection of apoptosis, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) was used. Formalin-fixed tissue sections were stained using an ApopTag Plus Peroxidase In Situ Apoptosis detection kit (Chemicon) according to the manufacturer’s instructions. For scoring, 100 randomly selected glomeruli were examined, and 20 randomly selected fields of renal tubules in the cortical area were examined by light microscopy at the magnification of ×400, as described previously (24). The number of phospho-NF-κBp65, CD3+, F4/80+, CD11b+, and apoptotic cells was counted in glomeruli and expressed as cells/glomerular cross section (24).

Flow Cytometry

Splenocytes from the mice were treated with Tris-buffered ammonium chloride to lyse erythrocytes, washed, and resuspended in RPMI 1640 supplemented with 10% fetal calf serum in HEPES buffer, 1 mM potassium chloride to lyse erythrocytes, washed, and resuspended in RPMI 1640 supplemented with 10% fetal calf serum in HEPES buffer, and then splenocytes from the mice, prepared as described as above, were added in triplicate to the wells (5 × 10⁵ cells in 200 μl/well) for 48 h, at which time the cultures were pulsed with 1 μCi of [³H]methyl thymidine (Amersham Pharmacia Biotech), harvested 16 h later, and the incorporated [³H]methyl thymidine was measured using TopCount (Packard, PerkinElmer, Boston, MA) as described previously (24).

Statistical analysis. Values are means ± SE. Comparison between two groups was performed using Student’s t-test. A P value < 0.05 was considered statistically significant.

RESULTS

DCR3 Ameliorates Proteinuria and Prevents Renal Function Impairment and Aggravation of Renal Pathology in Prg-IgAN Mice

Compared with empty vector-treated Prg-IgAN mice (disease control), DCR3-treated Prg-IgAN mice showed lower proteinuria at days 14 and 21 after disease induction (Table 1). They also showed improved renal function on days 14 and 21, as demonstrated by significantly lower BUN levels (Table 1), although there was no significant difference in Cr levels between the treated, disease control, and normal control mice.

By light microscopy, although the disease control mice showed only mild mesangial proliferation at day 3 (Fig. 2, A and B), at days 14 and 21 they showed marked glomerular proliferation (Fig. 2, A and B), focal, but intense, crescent formation (Fig. 2, A and C), focal glomerulosclerosis (Fig. 2, A and D), and characteristic periglomerular interstitial inflammation (Fig. 2, A and E). In contrast, the development of these progressive renal lesions was markedly inhibited in DCR3-treated Prg-IgAN mice (proliferation: day 3, P < 0.05, days 14 and 21, P < 0.01; crescent: days 14 and 21, P < 0.005; sclerosis: day 21, P < 0.005; inflammation: days 14 and 21, P < 0.005). Administration of DCR3 did not influence the amount or distribution of deposited IgA in the glomeruli in the disease control and DCR3-treated Prg-IgAN mice at any time after disease induction, as demonstrated by immunofluorescence (data not shown).

DCR3 Inhibits the Increase in T Cell Activity in Prg-IgAN Mice

We previously reported that T cells play a crucial role in initiating the progression of IgAN (6) and that DCR3 inhibits T cell proliferation in a mouse autoimmune nephritis model (24). We therefore tested whether DCR3 administration could negatively regulate 1) T cell activation, 2) T cell proliferation, and 3) T cell-mediated cytokine production in the Prg-IgAN model.

T cell activation. As shown in Fig. 3, A and B, the ratio of activated T cells in the spleen was significantly increased in disease control mice at day 3 compared with normal control mice (P < 0.005), then tended to return to normal at days 14

Table 1. Proteinuria and renal function

<table>
<thead>
<tr>
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<th>Day 3</th>
<th>Day 14</th>
<th>Day 21</th>
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<tr>
<td></td>
<td>Normal control</td>
<td>Prg-IgAN</td>
<td>Prg-IgAN+DCR3</td>
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<tr>
<td>24-h Urine protein</td>
<td>0.32 ± 0.10</td>
<td>0.56 ± 0.10</td>
<td>0.59 ± 0.13</td>
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<tr>
<td>BUN, mg/dl</td>
<td>28.18 ± 6.67</td>
<td>34.97 ± 4.23</td>
<td>37.53 ± 5.88</td>
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<tr>
<td>Cr, mg/dl</td>
<td>0.24 ± 0.07</td>
<td>0.2 ± 0.08</td>
<td>0.25 ± 0.06</td>
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Values are means ± SE. IgAN and Prg-IgAN, IgA nephropathy and progressive IgAN, respectively; BUN, blood urea nitrogen; Cr, creatinine. *P < 0.005 compared with normal control mice. †P < 0.05 compared with Prg-IgAN mice.
and 21. This increase was significantly inhibited by DCR3 administration at day 3 (P < 0.05). There was no detectable change in the ratio of activated B cells among the groups of mice (data not shown).

**T cell proliferation.** As shown in Fig. 3C, T cell proliferation was increased in Prg-IgAN mice at day 3 (P < 0.01), then returned to basal levels, and this increase was significantly inhibited in DCR3-treated Prg-IgAN mice (P < 0.05).

**T cell-mediated cytokine production.** The polarity of T helper cells plays an important role in the development and progression of IgAN in humans and animal models (6, 10, 58). Using intracellular staining, we demonstrated that, compared with T cells from normal control mice, there was an increase in the ratio of T cells (CD3+ cells) in the spleen of Prg-IgAN mice expressing INF-γ at days 14 and 21 (Fig. 4A) and in those expressing IL-4 at day 14 (Fig. 4B) and that the increases at day 14 were significantly inhibited by DCR3 treatment.

**DCR3 Inhibits Renal T Cell and Macrophage Infiltration in Prg-IgAN Mice**

Renal infiltration of T cells (6, 58) and/or monocytes/macrophages (6, 55, 59) plays a pivotal pathogenic role in the progression of IgAN. As shown in Fig. 5, diffuse infiltration of T cells (CD3+) and monocytes/macrophages (CD11b+ or F4/80+) into the periglomerular region of the renal interstitium...
was seen in Prg-IgAN mice, beginning on day 14 and increasing up to day 21 when the mice were euthanized, and this effect was dramatically reduced by DCR3 administration \( P < 0.05 \) for all 3. Only a very few mononuclear leukocytes were seen in Prg-IgAN mice or DCR3-treated Prg-IgAN mice on day 3.

DCR3 Inhibits the Increase in Renal Proinflammatory Cytokine mRNA Levels in Prg-IgAN Mice

Proinflammatory cytokines, especially MCP-1 (6, 39, 55) and IL-6 (6), contribute to progression of IgAN. We therefore measured renal mRNA levels for both cytokines. Prg-IgAN mice showed a significant increase in both MCP-1 and IL-6 mRNA levels compared with normal control mice on days 3, 14, and 21 (Fig. 6, A and B), and the increases on days 14 and 21 were markedly inhibited by DCR3 treatment (both \( P < 0.05 \)), although there was no significant difference in mRNA levels between DCR3-treated and disease control mice at day 3. In parallel, an immunohistochemical study showed that MCP-1 and IL-6 protein levels were significantly increased in Prg-IgAN mice at days 14 and 21 and that these increases were markedly inhibited by DCR3 treatment (Fig. 6, C–F). There was some discrepancy between mRNA and protein expression levels, and it might be explained by the influence of posttranscriptional regulation and/or differences in mRNA and protein turnover rates (13, 16). Since the NF-κB-dependent proinflammatory pathway plays a critical role in the pathogenesis of Prg-IgAN (6), we examined NF-κB p65 activation by immunohistochemistry and found that the increase in NF-κB p65 activation in the kidney in disease control mice was significantly inhibited in DCR3-treated Prg-IgAN mice compared with disease control mice at days 14 and 21 (Fig. 7), although at day 3 there was no significant difference between the two groups of mice.

DCR3 Inhibits the Increase in Apoptosis in the Spleen and Kidney of Prg-IgAN Mice

Apoptosis is a tightly regulated process of programmed cell death, and its abnormal regulation is responsible for the severely damaged glomeruli in the progression of IgAN (9, 41, 44, 45). Since DCR3 has been shown to modulate apoptosis in renal cells (24), cultured lymphocytes (66), and some tumor cells (43), we examined whether inhibition of apoptosis was

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**Fig. 3.** T function in the spleen. A: immunofluorescence dot-plot pattern of activated T cells (CD3+CD69+). B: ratio of activated T cells. C: T cell proliferation induced by anti-CD3 antibodies measured by [3H]methyl thymidine analysis. Values are means ± SE for a group of 12 mice. +++P < 0.05, ++P < 0.01, and +++P < 0.005.

**Fig. 4.** Intracellular cytokine staining in the spleen. Flow cytometry was used to estimate ratio of T cells (CD3+ cells) expressing IFN-γ (A) or IL-4 (B). Values are means ± SE for a group of 12 mice. *P < 0.05, **P < 0.01, and ***P < 0.005.
involved in the inhibition of the acceleration and progression of Prg-IgAN by DCR3 overexpression. Using a TUNEL assay, a significant increase in apoptosis was seen in the spleen (Fig. 8, A and B) and kidney (Fig. 8, C and D), including glomerular and tubulointerstitial compartments, in Prg-IgAN mice compared with normal control mice at days 14 and 21, and these increases were significantly inhibited by DCR3 treatment.

DCR3 Inhibits the Increase in Renal Expression of Fibrosis-Related Genes in Prg-IgAN Mice

Prg-IgAN tends to evolve rapidly to glomerular sclerosis and interstitial fibrosis (56, 59). We therefore evaluated the effects of DCR3 gene therapy on renal fibrosis in the Prg-IgAN model, focusing on Col-IV and Fn. As shown in Fig. 9, A and B, a marked increase in renal levels of both mRNAs was observed in Prg-IgAN mice compared with normal controls at days 14 (Col-IV mRNA) and 21 (both mRNAs), as demonstrated by real-time PCR (Col-IV: days 14 and 21, both P < 0.005; Fn: day 21, P < 0.005), and both increases were markedly inhibited by DCR3 (Col-IV: days 14 and 21, both P < 0.005; Fn: day 21, P < 0.01).

Again, greatly enhanced expression of the proteins encoded by both fibrosis-related genes was demonstrated by immunohistochemistry in empty vector-treated Prg-IgAN mice compared with normal control mice at days 14 and 21, and both increases were significantly reduced by DCR3 treatment (Fig. 9, C–F).

DISCUSSION

In the present study, we examined whether in vivo overexpression of DCR3 could prevent the progression of IgAN in a mouse Prg-IgAN model, the most remarkable pathogenetic feature of which is the dramatic systemic activation of T cells and the NF-κB-dependent inflammatory pathway. We found that in this Prg-IgAN model, characterized by clinically accelerated immunological and pathological features similar to those in the progression phase in IgAN patients, systemic overexpression of DCR3 protein by hydrodynamics-based gene delivery protected the mice from the development of diffuse proliferation, focal crescent formation, and sclerosis in the glomerulus and from renal interstitial (especially periglomerular) inflammation. Further studies revealed that negative regulation of T cell activation, blocking of the NF-κB-dependent inflammatory pathway, and suppression of apoptosis in the kidney may account for the favorable effects of DCR3 plasmid administration in the Prg-IgAN model. Since DCR3 administration has been shown to have beneficial effects on both crescentic glomerulonephritis (24) and the Prg-IgAN
Fig. 6. Renal proinflammatory cytokine expression. A and B: Real-time PCR was used to measure MCP-1 mRNA (A) or IL-6 mRNA (B). C and E: immunohistochemistry (IHC) results for staining of the kidney for MCP-1 (C) or IL-6 (E). Original magnification, ×400. D and F: scoring of IHC results. Values are means ± SE for a group of 12 mice. *P < 0.05, **P < 0.01, and ***P < 0.005.
model with crescent formation, the treatment with DCR3 might not be specific for IgAN and probably works for any immune or pauci-immune glomerulonephritis with crescent formation and heavy T cell infiltration.

Abnormal “hyperfunction” of T cells is considered to play a crucial role in the transformation of IgAN into its progressive form (Prg-IgAN) (6, 12, 47, 58). When we administered the DCR3 plasmid to Prg-IgAN mice, we observed significant inhibition of the increase in T cell activation (Fig. 3, A and B) and T cell proliferation (Fig. 3C) seen in these mice. In this regard, DCR3 might 1) inhibit the percentage of T cells expressing IFN-γ and IL-4; 2) interact with LIGHT to down-regulate the alloresponsiveness of T cells; 3) avoid immune attack through lymphocyte infiltration; and 4) modulate the differentiation and function of macrophages and dendritic cells (4, 21, 24, 43), which might prevent the T cell-mediated deterioration of IgAN. Our data also showed that DCR3 administration also greatly reduced the IFN-γ+CD3+ T cell ratio in the spleen of Prg-IgAN mice (Fig. 4A). It should be noted that INF-γ contributes to the progression of IgAN (28, 29, 37), and the effect of DCR3 on INF-γ expression could, in part, explain its favorable effects on the renal condition in the Prg-IgAN mice. Besides, DCR3 has also been shown to lower IFN-γ production in autoimmune encephalomyelitis in vivo (8) and in vitro (40), immunological tolerance for hepatocellular carcinoma (7), and T cell responses to alloantigens (66). In addition, inflammatory events, such as inflamed intestinal epithelia (25), and patients with bacterial infections (26) have been shown to have increased DCR3 protein production mediated by Toll-like receptor stimulation, suggesting that the latter might play a role in infectious conditions. It may be worth exploring whether the favorable effects of DCR3 administration on the Prg-IgAN model might partly result from a Toll-like receptor-dependent pathway. Our data support the idea that blockade of T cell activity could be a key mechanism by which DCR3 prevents progressive renal injury in IgAN. On the other hand, Th1- and Th2-related cytokines are considered to negatively regulate one another (23). Several studies have proposed a Th2 predominance in IgAN (11, 42, 50), but recent observations have confirmed a Th1 predominance in IgAN (20, 33, 54). In the present study, although intracellular levels of both IFN-γ and IL-4 in the spleen were elevated in the disease control mice, DCR3 administration resulted in a decrease in the intracellular levels of both of the cytokines in the DCR3-treated mice (Fig. 4). Whether the change in Th1/Th2 balance by DCR3 administration is a major mechanism responsible of the beneficial effects may be worth further investigation. However, these findings cannot exclude a possible pathogenic role.
of Th17 cells in this Prg-IgAN model, if further study in dissecting the pathway involving Th17 cells in the spleen can be performed, because of the role of these T cells in immune complex glomerulonephritis (51, 57).

Blocking of renal mononuclear leukocytic infiltration by DCR3 might also be involved in its favorable effects in preventing the development of Prg-IgAN. Clinically, the proliferative and crescentic forms of IgAN account for up to 30% of cases of IgAN and are characterized by nephrotic range proteinuria, accelerated hypertension, and an accelerated trend toward end-stage renal disease (29). The intraglomerular cellular structures of crescents are formed partly by proliferation of parietal epithelial cells and partly by mononuclear infiltrates (1, 31). In this regard, MCP-1 is considered to be actively involved in the progression of IgAN (6, 39, 55), and deletion of T cells and/or macrophages attenuates severe glomerulonephritis, such as crescentic glomerulonephritis (30, 32), suggesting an essential role for mononuclear leukocytes in the pathogenesis of the acceleration or deterioration of glomerulonephritis. We showed that DCR3 administration to Prg-IgAN mice resulted in reduced renal levels of MCP-1 protein (Fig. 6, C, and D), periglomerular and interstitial mononuclear leukocyte infiltration, and glomerular crescents. Furthermore, we previously demonstrated that DCR3 can markedly inhibit the local production of MCP-1 in the kidney in an autoimmune crescentic glomerulonephritis model in mice (24).

IL-6 has been implicated in the pathogenesis of Prg-IgAN (6, 62). In agreement with these reports, the development of

Fig. 8. Apoptosis in the spleen and kidney. A and C: terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) results for the spleen (A) and kidney (C). Original magnification, ×400. Arrows indicate positively stained cells. B and D: scoring of apoptosis-positive cells in the spleen (B) and kidney (D). Values are means ± SE for a group of 12 mice. **P < 0.01 and ***P < 0.005.
this Prg-IgAN model shows a strong pathogenic correlation with IL-6 levels. Our data (Fig. 6, B, E, and F) and those of others (40) clearly demonstrate that DCR3 administration can result in a significant reduction in IL-6 mRNA and protein levels in vivo, suggesting that this effect could be crucial for DCR3 to protect against renal injury in Prg-IgAN.

Another mechanism for the protective effects of DCR3 in the Prg-IgAN model is the prevention of local apoptosis in the...
kidney. Apoptosis is considered to contribute to the evolution of IgAN into its progressive form (9, 41, 45, 60), and, in agreement with this, we showed that blocking of apoptosis by DCR3 gene therapy in both the glomerular and tubulointerstitial compartments of the kidney was associated with lower histopathological severity of renal damage (Figs. 2 and 8, C and D). Antiapoptotic effects are generally considered to be beneficial in preventing IgAN (30, 45), although there is a report that enhancement of apoptosis is beneficial in a glomerular disorder model by inhibiting mesangial cell proliferation (36). Thus inhibition of apoptosis in the kidney might partly explain the beneficial effects of DCR3 administration on Prg-IgAN.

Interestingly, overproduction of DCR3 protein in transgenic mice (20–80 ng/ml in serum) has been shown to result in a systemic lupus erythematosus-like syndrome after 6 mo of age by inhibition of T cell death or the survival of abnormal self-cross-reactive T cells (17, 18). In our study, there was no evidence of exacerbation or development of the autoimmune-like conditions observed in the Prg-IgAN mice throughout the experiment, although DCR3 was administered for a relatively short period (3 doses of DCR3 plasmids within 21 days starting on day −1). However, in our previous report (24), beneficial effects in vivo were observed even when DCR3 plasmid treatment was given for up to 2 mo and serum levels of DCR3 were 100–170 ng/ml. No systemic lupus erythematosus-like features were described in DCR3 transgenic mice with a low serum level of DCR3 (4.7 ng/ml) (21). Besides, localization of the DCR3 plasmid in the kidney would provide strong evidence for its direct role in preventing deterioration of the model of IgAN. We suggest that DCR3 might have different biological functions in vivo depending on the cumulative dose. The role of the DCR3-induced reduction in splenic apoptosis in Prg-IgAN mice remains uncertain, although it was found to result in beneficial effects on an autoimmune crescentic glomerulonephritis mouse model in our previous study (24). In the present study, we delivered the DCR3 plasmids to the mice before the development of Prg-IgAN and demonstrated a preventive effect on their kidneys. Besides, it is likely that if the DCR3 treatment was started after the induction of IgAN in the mice and there might be therapeutic effects on the them, then the value of this gene therapy would be greatly enhanced, because in most clinical settings renal disease would have already been developed before the start of medical treatment to the patients. Meanwhile, it is worth performing an experiment after the stopping of DCR3 delivery and to see the effect on the severity of the renal disease to determine whether the DCR3 treatment has to be maintained to achieve a beneficial outcome.

In conclusion, our data show that administration of DCR3 plasmids by hydrodynamics-based gene delivery prevents the development of Prg-IgAN. We provide evidence that systemic modulation of T cell activation/proliferation might play a major role in the favorable effects of DCR3 in this model. Prevention of mononuclear leukocyte infiltration and apoptosis locally in the kidney may also be directly involved in the effects of DCR3 on this progressive type of experimental IgAN.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


