Effect of Hydrodynamics-Based Gene Delivery of Plasmid DNA Encoding Interleukin-1 Receptor Antagonist-Ig for Treatment of Rat Autoimmune Myocarditis

Possible Mechanism for Lymphocytes and Noncardiac Cells

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Background—Interleukin-1 (IL-1) is a powerful and important cytokine in myocarditis. The purpose of this study was to evaluate the effect and possible mechanism of hydrodynamics-based delivery of the IL-1 receptor antagonist (IL-1RA)-immunoglobulin (Ig) gene for treatment of rat experimental autoimmune myocarditis (EAM).

Methods and Results—On the day after immunization, rats were transfected with either pCAGGS encoding IL-1RA-Ig or pCAGGS encoding Ig alone. On day 17, IL-1RA-Ig gene therapy was effective in controlling EAM, as monitored by a decreased ratio of heart weight to body weight, reduced myocarditis areas, reduced gene expression of atrial natriuretic peptide in hearts, and improved cardiac function in echocardiographic and hemodynamic parameters. Examination of the expression of IL-1-related genes in purified cells from EAM hearts suggested that ectopic IL-1RA-Ig–acting target cells were αβT cells and noncardiomyocytic noninflammatory cells such as fibroblasts, smooth muscle cells, and endothelial cells. Therefore, we examined the effect of serum containing IL-1RA-Ig on the expression of immune-relevant genes within noncardiomyocytic cells cultured from EAM hearts or concanavalin A–stimulated lymphocytes derived from lymph nodes in EAM-affected rats. The expression of immunologic molecules (prostaglandin E synthase, cyclooxygenase-2, and IL-1β) in cultivated noncardiomyocytic cells and Th1 cytokines (IL-2 and IFN-γ) in lymphocytes was significantly decreased by the serum containing IL-1RA-Ig.

Conclusions—EAM was suppressed by hydrodynamics-based delivery of plasmid DNA encoding IL-1RA-Ig. In addition, IL-1RA-Ig suppressed gene expression of prostaglandin synthases and IL-1 in noncardiomyocytic cells and Th1 cytokines in lymphocytes. (Circulation. 2005;111:1593-1600.)

Key Words: cardiomyopathy, dilated cytokines sialoglycoproteins myocarditis prostaglandins

Received May 26, 2004; revision received November 5, 2004; accepted November 10, 2004.

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Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000160348.75918.CA
lular IL-1RA (iIL-1RA). IL-1RA functions as an antagonist by competitively binding to IL-1RI. IL-1RA cannot transduce intracellular signals because it is unable to bind to IL-1Racp. IL-1RA–based therapies are being evaluated for a variety of diseases.

The purpose of the present study was to investigate whether IL-1RA transduction ameliorated EAM and by what mechanisms this therapy occurred. Hydrodynamics-based gene transfer via the rapid tail vein injection of a large volume is more efficient than delivery by intramuscular injection with electroporation. This method can retrogradely deliver plasmid DNA predominantly into hepatocytes via hepatic vein. Moreover, chimeras with immunoglobulin (Ig) facilitate elevated concentration levels. In this study, we examined the efficacy of hydrodynamics-based delivery of plasmid DNA encoding an IL-1RA-Ig chimera.

Methods

Animals

Seven-week-old male Lewis rats were purchased from Charles-River Laboratories, Japan (Atsugi, Kanagawa, Japan) and were maintained in our animal facilities. Throughout the studies, all the animals were treated in accordance with the guidelines for animal experiments as laid out by our institute.

Induction of EAM

Cardiac myosin was prepared from the ventricular muscle of porcine hearts as previously described. To produce EAM, each rat was immunized on day 0 with 0.2 mL emulsion containing cardiac myosin with an equal volume of Freund’s adjuvant by a single subcutaneous injection in both footpads.

In Vivo Treatment of EAM With Plasmid DNA Encoding IL-1RA-Ig Gene

We first constructed the plasmid vector pCAGGS-Ig-glucagon (Glu)-tag, containing Swa and Norl restriction sites, via polymerase chain reaction (PCR) amplification. For this purpose, initial PCR products were generated from rat spleen cDNA using KOD Plus DNA polymerase (Toyobo) and the following primers: 5'-gaGAATTCATTTAAATgaagaGCGGCCGCcgccagaaactgtg-3' (contains both Swa and Norl restriction sites) and 5'-tcacacgtcacaacattttggtttaacggagagcact-3'. The final PCR product inserts were then amplified from the diluted products of the first PCR reaction with the following primers: 5'-gaGAATTCATTTAAATgagaGAATTCgcggttactccca-3' (as before) and 5'-gagaGAGAATTCgagtttcctcaacacccgcgtgcccagaaactgtg-3'. Finalized PCR products were inserted into the pCAGGS vector using EcoRI sites. Escherichia coli JM109 competent cells were then transformed, and recombinant plasmids were isolated by use of a Quantum Prep Plasmid Maxiprep kit (Bio-Rad Laboratories). To construct the control plasmid, pCAGGS- rat signal peptide (SP)-Ig-Glu-tag, the SP region of secretory leukocyte protease inhibitor was amplified from rat spleen cDNA using the primers 5'-gaGAATTCATTTAAATgagaGAATTCgcggttactccca-3' and 5'-gaGAATTCATTTAAATgagaGAATTCgcggttactccca-3', followed by insertion into pCAGGS-Ig-Glu-tag using Swa and Norl sites. Recombinant plasmids were isolated as described above.

Plasmid DNA Injection Techniques

Nineteen rats were divided into 2 groups, the pCAGGS-IL-1RA-Ig group (IL-1RA-Ig group; n=10) and the pCAGGS-SP-Ig group (SP-Ig group; n=9). Rats were injected with 800 µg pCAGGS-mouse IL-1RA-Ig-Glu-tag or pCAGGS-SP-Ig-Glu-tag via the tail vein within 15 seconds (receiving ~80 mL/kg body weight) on day 1.

Plasmid Chimeric Glucagons-Tag Protein Measurement

Blood samples were taken on days 2, 5, 8, 12, and 17. Glucagon concentrations were measured with a glucagon radioimmunoassay kit (Daichi Radioisotope Laboratories). Chimeric protein concentrations in blood were calculated with Glu-tag. To observe the relationship of them and gene expression in liver, the livers were harvested on days 2, 5, 8, 12, and 17 after injection of pCAGGS-IL-1RA-Ig-Glu-tag into normal rats (n=4, respectively), and transgene expressions were examined by real-time reverse-transcriptase (RT) PCR using the following primers: 5'-tcagctgagctgatttaccc-3' (726 to 748 bases in pCAGGS) and 5'-atacgatgaattttaccc-3' (316 to 339 bases in mouse sIL-1RA).

Evaluation of Echocardiography and Hemodynamic Parameters

On day 17, echocardiography was performed with a 7.5-MHz probe (SSD-630, Aloka ECHO camera). Left ventricular (LV) internal diameter in end diastole and end systole, interventricular septal thickness, LV posterior wall thickness, pericardial effusion (PE) under LV posterior wall thickness, and LV fractional shortening were calculated from M-mode echocardiograms over 3 consecutive cardiac cycles.

The hemodynamic parameters were measured after echocardiography. Mean arterial pressure was recorded through a catheter introduced into the right femoral artery. Central venous pressure (was recorded through a catheter introduced into the confluence of the vena cava with the right jugular vein. A catheter-tip transducer was inserted into the left ventricle from the right carotid artery to measure the peak left ventricular pressure and left ventricular end-diastolic pressure. The rates of intraventricular pressure rise and decline (±dP/dt) were measured with a differential amplifier. Heart rate was calculated from ECGs. All hemodynamic parameters were recorded on a thermostylus recorder after a stabilizing period of 10 minutes.

Evaluation of Histopathology

Heart and body weights were measured, and the ratio of heart weight to body weight (g/g) was calculated. Several transverse sections were cut from the midventricle slice and stained with Azan-Mallory. The myocarditis area of each specimen was determined with a color image analyzer (Mac SCOPE version 2.6, Mitani Corp).

Measurement of Atrial Natriuretic Peptide mRNA Levels

To measure mRNA levels of atrial natriuretic peptide (ANP), a heart failure marker, total RNA was isolated from the apical one third of the heart on day 17. The absolute copy number of ANP mRNA was measured by quantitative real-time RT-PCR.

Gene Expression of IL-1 Family in EAM Hearts

To evaluate crosstalk between members of the IL-1 family, the mRNA levels of IL-1α, IL-1β, IL-1RI, IL-1RII, IL-1Racp, sIL-1RA, or total IL-1RA (sIL-1RA+iIL-1RA) in both isolated and purified cells from EAM hearts were measured. On day 18, cardiomyocytes and the other cells in the hearts of EAM rats were isolated after collagenase perfusion treatment for 20 minutes with a Langendorff apparatus as reported previously. Isolated cells, while maintained in an isotonic buffer, were separated serially through stainless steel sieves into cardiomyocytes and the other cells. Because the inflammatory cells are almost CD11b+ cells (macrophages/dendritic cells/granulocytes) and αβT cells, the other cells without cardiomyocytes were separated into αβT cells, CD11b+ cells, and noncardiomyocytes.
TABLE 1. Absolute Copy Numbers of Specific Cell Marker mRNA in Cultivated Cells

<table>
<thead>
<tr>
<th>mRNA Marker</th>
<th>NC Cells (n=6)</th>
<th>Lymph Node Cells (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen type III</td>
<td>149 000 ±215 000</td>
<td>149 000 ±215 000</td>
</tr>
<tr>
<td>Collagen type III</td>
<td>149 000 ±215 000</td>
<td>149 000 ±215 000</td>
</tr>
<tr>
<td>Calponin</td>
<td>23 400 ±11 000</td>
<td>ND</td>
</tr>
<tr>
<td>CD11b</td>
<td>76 000 ±25 000</td>
<td>ND</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>194 ±31</td>
<td>260 ±56</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

TABLE 2. List of Primers for Quantitative RT-PCR

<table>
<thead>
<tr>
<th>Sense Primer</th>
<th>Antisense Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP</td>
<td>5'-gtcccaatctcgtgatcctg-3'</td>
</tr>
<tr>
<td>α-Cardiac myosin</td>
<td>5'-ctttcattcagcatctcag-3'</td>
</tr>
<tr>
<td>CD3</td>
<td>5'-gtctgactgtagtgctgctg-3'</td>
</tr>
<tr>
<td>CD11b</td>
<td>5'-ctgtgagaggactgctgctg-3'</td>
</tr>
<tr>
<td>Collagen type III</td>
<td>5'-cttttccccttctcttctct-3'</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>5'-ctttttcccttctcttctct-3'</td>
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<tr>
<td>Calponin</td>
<td>5'-ctttttcccttctcttctct-3'</td>
</tr>
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<td>IL-1α</td>
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<td>IL-1β</td>
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<td>IL-1RII</td>
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<td>IL-1Rap</td>
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</tr>
<tr>
<td>sIL-1RA</td>
<td>5'-ctttttcccttctcttctct-3'</td>
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<tr>
<td>Total IL-1RA</td>
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<td>IL-2</td>
<td>5'-ctttttcccttctcttctct-3'</td>
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<tr>
<td>IFN-γ</td>
<td>5'-ctttttcccttctcttctct-3'</td>
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<tr>
<td>PGES</td>
<td>5'-ctttttcccttctcttctct-3'</td>
</tr>
<tr>
<td>Cox-2</td>
<td>5'-ctttttcccttctcttctct-3'</td>
</tr>
<tr>
<td>γ-Actin</td>
<td>5'-ctttttcccttctcttctct-3'</td>
</tr>
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</table>
With the same primer pair used for making the plasmid and a LightCycler-FastStart DNA Master SYBR Green I kit (Roche). After an initial denaturation step of 10 minutes at 95°C, a 3-step cycling procedure (denaturation at 95°C for 10 seconds, annealing at 62°C for 10 seconds, and extension at 72°C for 13 seconds) was used for 40 cycles. The absolute copy numbers of particular transcripts were calculated by LightCycler software using a standard curve approach.4 cDNA from cultivated cells was then subjected to quantitative RT-PCR analysis, with the level of β-actin mRNA acting as an internal control.

Statistical Analysis
Statistical assessment was performed by unpaired Student t test or 1-way ANOVA and Bonferroni multiple comparison test. The differences were considered significant at P<0.05. Ratio of heart weight to body weight, myocarditis area, echocardiography and hemodynamic parameters, data obtained from quantitative RT-PCR, and concentration of IL-1RA-Ig-Glu-tag and Ig-Glu-tag were expressed as mean±SEM.

Results
Effect of In Vivo Treatment With Plasmid DNA Encoding IL-1RA-Ig Gene
The heart to body weight ratios in the IL-1RA-Ig group were significantly lower than those of the SP-Ig group (mean±SEM, 0.53±0.03% versus 0.59±0.05%; P=0.0043) (Figure 1A). The inflammatory area in the IL-1RA-Ig group was significantly smaller than that observed in the SP-Ig group (22.3±4.8% versus 34.6±6.8%, P=0.0003) (Figure 1B). Expression of ANP mRNA (a heart failure marker) was significantly lower in heart tissues of the IL-1RA-Ig group than those of controls (7.13×10^6–3.62×10^6 versus 31.6×10^6–24.2×10^6 copy/total RNA µg; P=0.0084) (Figure 1C).

Echocardiographic and Hemodynamic Parameters
As shown in Table 3, the LV fractional shortening and the absolute value of +dP/dt or −dP/dt in IL-1RA-Ig group were significantly larger than in SP-Ig group. LV end-systolic diameter, LV posterior wall thickness, PE, LV end-diastolic pressure, and central venous pressure were significantly smaller in the IL-1RA-Ig group than in SP-Ig group.

Time Course of IL-1RA-Ig-Glu-Tag Protein Levels
Plasma IL-1RA-Ig-Glu-tag protein levels in the IL-1RA-Ig group were found to increase, peaking at 23.21±8.52 nmol/L (mean±SEM) on day 2, and gradually decrease to 5.56±2.70 nmol/L on day 5, 1.64±0.63 nmol/L on day 8, 0.85±0.45 nmol/L on day 12, and 0.22±0.07 nmol/L on day 17. The plasma Ig-Glu-tag protein levels in the SP-Ig group were seen

TABLE 3. Echocardiographic and Hemodynamic Parameters

<table>
<thead>
<tr>
<th></th>
<th>IL-1RA-Ig (n=7)</th>
<th>SP-Ig (n=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDd, mm</td>
<td>5.41±0.05</td>
<td>5.48±0.29</td>
<td>0.81</td>
</tr>
<tr>
<td>LVESd, mm</td>
<td>3.01±0.17</td>
<td>3.81±0.24</td>
<td>0.018</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>1.84±0.05</td>
<td>1.96±0.12</td>
<td>0.33</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>1.89±0.17</td>
<td>2.24±0.27</td>
<td>0.012</td>
</tr>
<tr>
<td>PE, mm</td>
<td>1.71±0.47</td>
<td>3.67±0.49</td>
<td>0.016</td>
</tr>
<tr>
<td>LVFS, %</td>
<td>44.4±2.9</td>
<td>30.4±2.4</td>
<td>0.0039</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>354.9±14.3</td>
<td>381.5±19.8</td>
<td>0.29</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>1.73±0.26</td>
<td>3.93±0.55</td>
<td>0.0029</td>
</tr>
<tr>
<td>AP, mm Hg</td>
<td>78.4±2.52</td>
<td>72.6±1.72</td>
<td>0.094</td>
</tr>
<tr>
<td>LVP, mm Hg</td>
<td>91.1±3.53</td>
<td>85.9±2.08</td>
<td>0.244</td>
</tr>
<tr>
<td>EDP, mm Hg</td>
<td>11.7±1.3</td>
<td>18.4±2.24</td>
<td>0.0218</td>
</tr>
<tr>
<td>+dP/dt, mm Hg/s</td>
<td>4459±243</td>
<td>3638±178</td>
<td>0.0226</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td>−5547±352</td>
<td>−4129±202</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

LVEDd indicates LV end-diastolic internal diameter; LVESd, LV end-systolic internal diameter; IVS, interventricular septal thickness; LVPW, LV posterior wall thickness; LVFS, LV fractional shortening; HR, heart rate; CVP, central venous pressure; AP, mean blood pressure; LVP, peak LV pressure; EDP, LV end-diastolic pressure; +dP/dt, maximum dP/dt; and −dP/dt, minimum dP/dt.

Result are expressed as mean±SEM.
to increase, peaking at 22.75±2.34 nmol/L on day 2, and gradually decrease to 19.48±1.62 nmol/L on day 5, 17.50±2.50 nmol/L on day 8, 15.59±3.56 nmol/L on day 12, and 7.45±1.88 nmol/L on day 17 (Figure 2A). The expressions of IL-1RA-Ig-Glu-tag transferred into normal rat liver by hydrodynamics-based gene delivery were similar to plasma IL-1RA-Ig-Glu-tag protein levels (Figure 2B). It has been reported that IL-1RA (1 to 10 ng/mL, 0.05 to 0.5 nmol/L) suppresses the production of PGES from rat astrocytes stimulated by lipopolysaccharide in vitro.28 These results indicated that continuous effective delivery of IL-1RA-Ig protein for >16 days can be achieved in rats by hydrodynamics-based transfection.

Gene Expression of IL-1 Family in Purified Cells From EAM Hearts
Both IL-1α and IL-1β were strongly expressed in CD11b+ cells. The IL-1RI gene was strongly expressed in NCNI cells (the cell fraction containing mainly fibroblasts, smooth muscle cells, and endothelial cells) and αβT cells, whereas the IL-1RII gene was found to be markedly expressed in CD11b+ cells. The IL-1Racp gene was strongly expressed in both CD11b+ and NCNI cells and was moderately expressed in αβT cells. Both total IL-1RA and sIL-1RA were detected in CD11b+ cells. These results suggested that IL-1, produced mainly by CD11b+ cells, acted on NCNI and αβT cells by binding to IL-1RI and transduced intracellular signals by forming with IL-1Racp. On the other hand, CD11b+ cells also produced native IL-1RA and IL-1RII, potentially suppressing the action of IL-1 (Figure 3).

Expression of Immunologic Molecules in Cultivated Cells With Serum Containing IL-1RA-Ig
IL-1RA-Ig-containing serum significantly reduced expression of PGES (mean±SEM, 1.22±0.22 versus 1.94±0.17; P<0.0001), Cox-2 (0.93±0.24 versus 2.00±1.05; P=0.0092), and IL-1β (3.91±1.22 versus 11.25±2.16; P<0.0001) at the mRNA level in cultivated NC cells (the cell fraction containing mainly fibroblasts, smooth muscle cells, and CD11b+ cells) (Figure 4). In addition, IL-1RA-Ig-containing serum significantly reduced expression of the IL-2 (0.0004±0.0001 versus 0.0310±0.0090; P<0.0001) and IFN-γ (0.0065±0.0036 versus 0.0461±0.0385; P=0.0091) genes in cultivated lymphocytes (Figure 5).

Discussion
In the present study, we demonstrated that hydrodynamics-based delivery of plasmid DNA encoding the IL-1RA-Ig gene ameliorated EAM. IL-1RA-Ig–affecting cells were thought to be NCNI cells (fibroblasts, smooth muscle cells, and endothelial cells) and αβT cells because IL-1RI and IL-1Racp were found in them. Endogenous IL-1α and IL-1β produced mainly by CD11b+ cells, especially secreted IL-1β, influence these surrounding cells in a paracrine manner. IL-1RA-Ig, generated after gene transfer, appears to inhibit the IL-1–induced reactions of these cells and ameliorates EAM.

The functions of IL-1 with respect to cell regulation are varied.29 In this study, the effect of serum containing IL-1RA-Ig on the mRNA expression of various immunologic molecules in cultivated NC cells (mainly fibroblasts, smooth muscle cells, and CD11b+ cells) from hearts and lymphocytes from popliteal lymph nodes of EAM rats was investigated. The concentration of IL-1RA-Ig in cultivated cells was almost the same as that observed at the onset of myocarditis (day 10 to 11) and that reported by previous in vitro studies to be an effective dose.28,30 In this study, there was a 30- to 50-fold increase in the expression of PGES, Cox-2, and IL-1β mRNA in NC cells by IL-1 alone, but the increase was significantly reduced by serum containing IL-1RA-Ig. PGE2 produced by PGES plays an important role in inflammation and pain.31 In rheumatoid arthritis, the level of PGES detected...
at the inflammatory region is very high. Our study of PGES and Cox-2 gene expression in purified cells from EAM hearts indicated that they were produced mainly by NCNI cells and slightly by CD11b−/H11001 cells (data not shown). IL-1RA-Ig may inhibit the expression of the PGES and Cox-2 gene directly on NCNI cells via IL-1R. IL-1–induced IL-1 production has been shown in various cell types. IL-1RA-Ig may also inhibit IL-1 production directly on NCNI cells. However, because our study indicated that IL-1 was produced mainly by CD11b+ cells, IL-1RA-Ig may inhibit IL-1 production of CD11b+ cells indirectly via NCNI cells or αβT cells. Reduced PGES, Cox-2, and/or IL-1 production by NC cells in EAM hearts may be an effect resulting in improved myocarditis. On the other hand, in lymphocytes of popliteal lymph node, IL-2 and IFN-γ mRNA expression levels were significantly reduced by serum containing IL-1RA-Ig. In EAM, because our study indicated that IL-1 was produced mainly by CD11b+ cells, IL-1RA-Ig may inhibit IL-1 production of CD11b+ cells indirectly via NCNI cells or αβT cells. Reduced PGES, Cox-2, and/or IL-1 production by NC cells in EAM hearts may be an effect resulting in improved myocarditis. On the other hand, in lymphocytes of popliteal lymph node, IL-2 and IFN-γ mRNA expression levels were significantly reduced by serum containing IL-1RA-Ig. In EAM,

**Figure 3.** A through G, Absolute copy numbers of IL-1α, IL-1β, IL-1RI, IL-1RII, IL-1Racp, sIL-1RA, and total IL-1RA, respectively. Each cell was separated and purified from EAM heart on day 18. Error bars represent SEM. Statistical assessment was performed by 1-way ANOVA and Bonferroni multiple comparison test. Differences were considered significant at P<0.05.

**Figure 4.** Copy numbers of various immunologic molecules mRNA/copy numbers of γ-actin mRNA in NC cells cultivated from EAM hearts. A, PGES; B, Cox-2; C, IL-1β. Negative control cells were cultivated in medium without IL-1α and rat serum. SP-Ig+IL-1 cells were cultivated in medium with IL-1α and rat serum treated with pCAGGS-SP-Ig-Glu-tag; IL-1RA-Ig+IL-1 cells were cultivated in medium with IL-1α and rat serum treated with pCAGGS-mouse IL-1RA-Ig-Glu-tag. Error bars represent SEM. Statistical assessment was performed by 1-way ANOVA and Bonferroni multiple comparison test. Differences were considered significant at P<0.05.
which is a T cell–mediated disease. Th1 cytokines such as IL-2 and IFN-γ produced by CD4+ T cells are thought to play a central role. Therefore, inhibition of Th1 cytokine production by IL-1RA-Ig may improve EAM.

In human rheumatoid arthritis, it has been reported that Anakinra (a recombinant form of IL-1RA) suppressed progression of this disease, and treatment with IL-1RA has been investigated in various animal models. Therefore, the number of diseases in which IL-1RA has a therapeutic relevance may be extended in the near future. Human myocarditis is serious and often fatal despite intensive care. However, the cause of myocarditis is not usually evident. It was reported that gene therapy with IL-1RA expression plasmid was effective in the treatment of viral myocarditis and reduced virus titer in hearts. Here, we demonstrated that plasmid was effective in the treatment of viral myocarditis, which is a T cell–mediated disease, and IL-1RA-Ig and EAM may be effective for acute and fulminating myocarditis even if its cause is unknown.

Hydrodynamics-based gene delivery of plasmid DNA as used in this study is both inexpensive and highly effective in terms of facilitating gene expression. The concentration of IL-1RA-Ig in blood obtained by this method was higher compared with plasmid DNA transfection into muscle with in vivo electroporation. The producing cells to which plasmid DNA is transferred by this method are thought to be mainly hepatocytes. Plasmid DNA is thought to be delivered into hepatocytes by retrograde blood flow from hepatic veins. If plasmid DNA encoding IL-1RA-Ig can be directly transferred by this method are thought to be suitable for human clinical application. New developments of this gene therapy can be expected in the future.

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Circulation. 2005;111:1593-1600; originally published online March 28, 2005; doi: 10.1161/01.CIR.0000160348.75918.CA

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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