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Stimulation of FcγRI on Primary Sensory Neurons Increases Insulin-Like Growth Factor-I Production, Thereby Reducing Reperfusion-Induced Renal Injury in Mice

Naoaki Harada,* Juan Zhao,* Hiroki Kurihara,† Naomi Nakagata,‡ and Kenji Okajima*

Biological role(s) of FcγRI on mouse primary sensory neurons are not fully understood. Sensory neuron stimulation increases insulin-like growth factor-I (IGF-I) production, thereby reducing ischemia/reperfusion (I/R)-induced tissue injury in mice. In this study, we examined whether the Fc fragment of IgG (IgGFc) increases IGF-I production through sensory neuron stimulation, thereby reducing I/R-induced renal injury in mice. IgGFc increased the calcitonin-gene–related peptide (CGRP) release and cellular cAMP levels in dorsal root ganglion neurons isolated from wild-type (WT) mice, whereas, native IgG did not. Pre-treatment with anti-FcγRI Ab, a protein kinase A inhibitor KT5710, and a phospholipase A2 inhibitor 4-bromophenacyl bromide inhibited these effects induced by IgGFc. Administration of IgGFc enhanced increases of renal tissue levels of CGRP and IGF-I and reduced I/R-induced renal injury in WT mice. Increases of renal tissue levels of caspase-3, renal accumulation of neutrophils, and renal tubular apoptosis were inhibited by administration of IgGFc in WT mice subjected to renal I/R. Pre-treatment with anti–IGF-I Ab completely reversed these effects induced by IgGFc in WT mice. Administration of native IgG did not show any effects in WT mice subjected to renal I/R. None of the effects observed in WT mice was seen after IgGFc administration in CGRP-knockout mice and denervated WT mice. These observations suggest that activation of FcγRI by IgGFc may stimulate sensory neurons, thereby promoting IGF-I production, contributing to reduction of the reperfusion-induced renal injury via attenuation of inflammatory responses in mice. The Journal of Immunology, 2010, 185: 1303–1310.

Abbreviations used in this paper: ab, Ab; 4-BPB, 4-bromophenacyl bromide; BUN, blood urea nitrogen; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; IGF-I, insulin-like growth factor-I; IgGFc, Fc fragment of IgG; I/R, ischemia/reperfusion; IR, ischemia/reperfusion; MPO, myeloperoxidase; PLA2, phospholipase A2; P, phosphate; PKA, protein kinase A; PLAl, phospholipase A2; post, 15 min after reperfusion; pre, immediately prior to ischemia; SB, SB-366791; VR-1, vanilloid receptor-1; WT, wild-type; WT/denervation, denervated WT mice.

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We demonstrated that IGF-I reduces stress-induced gastric mucosal injury by decreasing gastric accumulation of neutrophils through inhibition of caspase-3 activation (18). These observations strongly suggest that stimulation of sensory neurons by activating FcγRI may increase IGF-I production, thereby reducing I/R-induced tissue injury by inhibiting neutrophil activation.

In this study, we analyzed the effect of IgGFc on CGRP release from dorsal root ganglion neurons isolated from wild-type (WT) mice in vitro and examined whether administration of IgGFc reduces reperfusion-induced renal injury by enhancing IGF-I production through inhibition of neutrophil activation in WT mice, CGRP-knockout (CGRP<sup>−/−</sup>) mice, and denervated WT mice.

Materials and Methods

Generation of α-calcitonin gene-related peptide-deficient mice

The generation of α-calcitonin gene-related peptide (αCGRP)-deficient (CGRP<sup>−/−</sup>) mice was described previously (19). The mouse CT/αCGRP genomic DNA was cloned from a BALB/c mouse genomic library in EMBL3 using synthetic oligonucleotide probes derived from the mouse CT/αCGRP DNA sequence. A 7.0-kb fragment containing exons 3 to 5 of the mouse CT/αCGRP gene was subcloned into pBluescript (Stratagene, La Jolla, CA). A targeting vector was constructed by replacing the 1.6-kb XbaI–XbaI fragment encompassing exon 5, which is specific for La Jolla, CA). A targeting vector was constructed by replacing the 1.6-kb XbaI–XbaI fragment encompassing exon 5, which is specific for αCGRP, with the neomycin resistance gene and flanking the thymidine kinase gene. This plasmid was linearized with NotI and introduced into 129/Sv-derived SM-1 ES cells by electroporation, after which the cells were selected in medium containing G418 (300 μg/ml). The targeted ES clones were injected into C57BL/6 mouse blastocysts to generate chimeric mice. Male chimeras were then crossed with C57BL/6 females, and germline transmission was achieved. Littermates obtained by breeding heterozygotes with the genetic background of the 129/SvxC57BL/6 hybrid were used for phenotypic analysis. Only males were used in this study.

Genotype determination of CGRP<sup>−/−</sup> pups

Genomic DNA was extracted from tails of mice as previously described (19) and was used for PCR analysis. PCR was performed using the external primers of the replaced gene fragment. The WT allele and the mutant allele gave different band sizes. Primer sequences and PCR conditions have been described (19).

Reagents

IgGFc was purified from pooled human plasma by Coomassie blue fractionation. To prepare the IgGFc, IgG of 10 mg/ml was dialyzed against 0.2 M MOPS in PBS containing 0.02% Tween-20 and 0.01 M EDTA. After inactivating papain by 0.03 mg/ml iodoacetamide and papain, IgGFc was purified by affinity chromatography using protein A-Sepharose, and Fe was isolated by gel filtration using Superose 12 (Pharmacia, 500 mg).

KT5720, an inhibitor of protein kinase A (PKA) (20), was purchased from Alexis (Basel, Switzerland). The 4-phenylbenzyl bromide (4-BPB), an inhibitor of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) (21), and SB366791, a specific inhibitor of VR-1 (22), were purchased from Sigma-Aldrich (St. Louis, MO). Anti-FcγRI Ab and anti-β2-microglobulin Ab were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were of analytical grade.

Isolation and culture of dorsal root ganglion neurons

Dorsal root ganglion (DRG) neurons from the lumbar, cervical, and thoracic region were dissected from WT mice as described previously (23). In brief, DRG neurons were placed in ice-cold sterile calcium- and magnesium-free Dulbecco’s PBS (Life Technologies, Grand Island, NY). Ganglia were chopped and incubated at 37°C for 15 min in Dulbecco’s PBS containing 20 U/ml papain (Worthington Biochemical, Lakewood, NJ). The tissue was then incubated at 37°C for 15 min in Dulbecco’s PBS containing 4 mg/ml collagenase type II (Worthington Biochemical). The tissue was incubated for an additional 30 min in Dulbecco’s PBS containing 4 mg/ml collagenase type II (Worthington Biochemical). The tissue was incubated for an additional 30 min in Dulbecco’s PBS containing 200 U/ml dispase I (Godo Shusei, Tokyo, Japan) at 37°C. Individual cells were then dissociated by trituration through a fine-polished Pasteur pipette. After centrifugation at 250g for 5 min, the resultant pellet was washed twice in serum-free Ham’s F-12 medium (Hyclone, Logan, UT). Cells were plated on a 60-mm poly styrene dish precoated with Vitrogen (Cohesion Technologies, Palo Alto, CA) in Ham’s F-12 medium containing 10% supplemented calf serum, 2 mM glutamine, and 50 μg/ml mouse 2.5 S nerve growth factor (Upstate Biotechnology, Lake Placid, NY). After 24 h, the culture medium was removed and replaced every 2 d.

Release of CGRP and cAMP measurement in cultured DRG neurons

After 5 d in culture, the medium was aspirated gently and washed with serum-free Ham’s F-12 medium. Cells were incubated with IgGFc (0.1, 1, and 10 mg/ml) and native IgG (1 mg/ml) for 30 min in Ham’s F-12 medium containing 1% supplemented calf serum without nerve growth factor. After incubation, supernatants were sampled and stored at −20°C for CGRP measurement. CGRP levels were determined using specific-enzyme immunoassay kit (SPL-BIO, Massy, France). Recent studies demonstrated that cAMP plays a critical role in CGRP release from sensory neurons by phosphorylating VR-1 through activation of PKA (24), and cAMP-dependent PKA activation is critically involved in CGRP production in DRG neurons (25). Therefore, we measured the intracellular cAMP levels in DRG neurons. We examined the effect of KT5720 on CGRP release from DRG neurons at a concentration of 10 μM, as described previously (19). After collection of supernatants, plates were placed on ice, media were removed, and cells were washed by ice-cold PBS. Thereafter, ice-cold 65% ethanol was added to each well and placed on ice. Ethanol solutions were collected and dried under nitrogen gas. Intracellular levels of cAMP were determined with an enzyme immunoassay kit (GE Healthcare, Buckinghamshire, U.K. according to the manufacturer’s instructions).

Mice model of renal I/R

Age-matched 7–10 wk old, 16–23 g male C57BL/6 WT and CGRP<sup>−/−</sup> mice were used in each experiment. The care and handling of the animals were in accordance with the National Institutes of Health guidelines. All experimental procedures described below were approved by the Nagoya City University Animal Care and Use Committee. During surgery, core temperature was monitored using a rectal probe and maintained with a heating pad and a Chemometr lamp at 35.5°C–37°C. The renal I/R protocol was performed as described previously (26), with some modifications. In brief, mice were anesthetized with an i.p. injection of pentobarbital sodium 50 mg/kg (Abbott Laboratories, Chicago, IL), followed by 0.1 mg/kg every 4 h and supplemented with an i.p. injection of buprenorphine 2 mg/kg every 4 h. To perform nephrectomy, a midline incision was made in each mouse and the right kidney was mobilized to allow the right renal artery to be ligated. To cause ischemia, the left pedicle was clamped with a noncrushing microvascular clamp for 45 min. Ischemia was confirmed by the blanching of the kidney. During the period of renal ischemia, the mice were covered with plastic wrap to prevent evaporation. After removal of the clamp, the left kidney was inspected for the restoration of blood flow and the contralateral kidney was removed. The wound was closed with a 3-0 silk suture. The animals were then returned to their cages and allowed free access to food and water. Sham-operated animals underwent the same operation but without clamping.

Sensory denervation by neonatal capsaicin treatment in WT mice

Sensory denervation by neonatal capsaicin administration was performed according to a previously described method (27, 28). Neonatal male WT mouse pups (C57BL/6, Nikon SL, Hamamatsu, Japan) were weighed and injected s.c. into the back with 50 mg/kg capsaicin on postnatal day 2. When these mice reached 7–10 wk old, they were tested for sensory denervation by applying 0.1 mg/ml capsaicin in saline solution to the eyes; absence of blinking or scratching confirmed sensory denervation. Capsaicin-treated mice that showed any blinking or scratching were excluded from the study.

Administration of various agents

IgGFc and native IgG were dissolved in sterile distilled water and were administered i.v. at a dosage of 100 mg/kg immediately prior to ischemia induction. SB366791 was dissolved in dimethyl sulfoxide (1%) with normal saline solution and was administered i.p. at a dosage of 500 μg/kg 30 min prior to ischemia (22). Anti-β2-microglobulin Ab was dissolved in sterile distilled water and was administered s.c. at a dosage of 50 μg/kg 30 min prior to ischemia (29).

Determination of renal CGRP levels

Tissue levels of CGRP were determined in animals by modification of the methods as described previously (20). The tissues were weighed and then homogenized in a polytron-type homogenizer (two times of 15 s) using 1 ml...
2 N acetic acid. The homogenates were bathed in 90°C water for 20 min and then centrifuged at 4500g for 10 min (4°C). CGRP was extracted from the supernatant by using reverse-phase C18 columns (GE Healthcare). Columns were prepared by washing with 5 ml methanol, followed by 10 ml water before use. The samples were applied onto the column, followed by washing with 20 ml 0.1% trifluoroacetic acid. CGRP was eluted with 3 ml 60% acetonitrile in 0.1% trifluoroacetic acid, and the solvent was evaporated under a stream of nitrogen gas. The concentration of CGRP was assayed by using a specific-enzyme immunosassay kit (SPI-BIO, Massey Cedex, France). The antiserum cross-reacts 100% with rodent α- and β-CGRP, according to the manufacturer’s data sheet. Results are expressed as micrograms of CGRP per gram of tissue.

**Determination of renal IGF-I level**

Renal levels of IGF-I were determined in animals as described previously (11). The hippocampus was minced and homogenized in a polytron-type homogenizer (two times of 15 s) using 1 ml 1 N acetic acid, according to the manufacturer’s instruction. The homogenates were then centrifuged at 4500g for 10 min. The supernatants were kept in a deep freezer at −80°C. The concentration of IGF-I was assayed by using a specific-enzyme immunosassay kit (Diagnostic Systems Laboratories, Webster, TX).

**Determination of renal myeloperoxidase activity**

Accumulation of neutrophils in the kidney was evaluated by measuring renal myeloperoxidase (MPO) activity at 6 h after reperfusion as described previously (16). In brief, the kidney was removed, weighed, and homogenized in 10% (w/v) 0.05 M phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide and sonicated for 20 s. After centrifugation (4500g for 20 min at 4°C), 0.1 ml of the supernatant was added to 0.55 ml 0.1 M phosphate buffer (pH 6.0) containing 1.25 mg/ml o-dianisidine and 0.05% hydrogen peroxide. After 5 min, changes in absorbance at 460 nm were measured using spectrophotometer. The activity of purified known human neutrophil MPO was used as the standard. One unit of MPO activity was defined as the amount of enzyme able to reduce 1 μmol peroxide/min. Results are expressed as units of MPO activity per gram of tissue.

**Determination of renal tissue levels of caspase-3**

Renal tissue levels of caspase-3 were determined at 6 h after reperfusion using a human active caspase-3 immunosassay kit (R&D Systems, Minneapolis, MN) as described previously (11, 30). In brief, the kidney was removed, weighed, and homogenized using 0.1 M phosphate buffer (pH 7.4) at 4°C. Homogenates were sonicated for 20 s and centrifuged (2000g for 10 min at 4°C). The results are expressed as micrograms of caspase-3 per gram of tissue.

**FIGURE 1.** Effects of IgGFc and native IgG on CGRP release (A) and cAMP levels (B) in DRG neurons isolated from WT mice in vitro and effects of anti-FcγRI Ab, KT5720, and 4-BPB on IgGFc-induced changes in CGRP release and cAMP levels in DRG neurons. DRG neurons were incubated with IgGFc (0.1, 1, and 10 mg/ml) or native IgG (1 mg/ml) for 30 min. DRG neurons were also incubated with IgGFc (1 mg/ml) in the presence of anti-FcγRI ab (20 ng/ml), KT5720 (10 μM), an inhibitor of PKA, or 4-BPB (10 μM), an inhibitor of PLXα, for 30 min. Data are expressed as means ± SD. *p < 0.01 versus media; †p < 0.01 versus IgGFc, ab, Ab.

**TUNEL stain**

To evaluate the extent of apoptosis in the kidney of mice subjected to renal I/R, samples were analyzed at 24 h after reperfusion with staining with in situ Apoptosis Detection Kit (Takara, Kyoto, Japan) system, which is a modification of Gavrieli’s method (11, 30). Slides were visualized by peroxidase method with diaminobenzidine and counterstained with H&E. Renal tubular apoptosis was identified by positive staining and counted in 10 high-power fields (HPFs) of a light microscope.

**Measurement of serum levels of blood urea nitrogen and creatinine**

Renal dysfunction was evaluated by measuring serum levels of blood urea nitrogen (BUN) and creatinine as previously described (16). Blood samples were taken from the abdominal aorta 24 h after reperfusion. Blood was collected in tubes and centrifuged at 2000g for 10 min. Serum levels of BUN and creatinine were measured by standard urease assays and picric acid reactions.

**Histopathological studies of the kidney**

The kidneys were removed 24 h after reperfusion, fixed in 10% formalin, embedded in paraffin, cut into 5-μm thick sections, and stained with H&E. The number of neutrophils per HPF (×400) were counted to assess the neutrophil infiltration into the kidneys. To compensate for variable tissue infiltration, the number of neutrophils in 10 given areas was counted and the means ± SD were calculated.

**Statistical analysis**

Data are expressed as the means ± SD. The results were compared using an ANOVA, followed by Scheffé’s post hoc test. A level of p ≤ 0.05 was considered statistically significant.

**Results**

**Effects of IgGFc, native IgG, and/or anti-FcγRI Ab on CGRP release and cAMP levels in sensory neurons isolated from WT mice in vitro**

To examine whether IgGFc and native IgG stimulate sensory neurons, we examined the effects of these substances on CGRP release from DRG neurons isolated from WT mice. IgGFc, at concentrations of 1 and 10 mg/ml, increased CGRP release from DRG neurons, whereas, native IgG, at a concentration of 1 mg/ml, did not (Fig.1A). Pretreatment with anti-FcγRI Ab did not affect...
CGRP release from DRG neurons; it reversed IgGFc-induced increase of CGRP release from DRG neurons (Fig. 1A).

cAMP has been shown to sensitize sensory neurons through PKA-dependent phosphorylation of VR-1 (31). To elucidate the mechanism by which IgGFc stimulates sensory neurons, we examined the effects of IgGFc and native IgG on cAMP levels in DRG neurons isolated from WT mice. As shown in Fig. 1B, IgGFc, at concentrations of 1 and 10 mg/ml, increased cAMP levels, whereas native IgG, at a concentration of 1 mg/ml, did not. Pretreatment with anti-FcγRI Ab did not affect baseline cAMP levels in DRG neurons; it reversed IgGFc-induced increases of cAMP levels in DRG neurons (Fig. 1B).

Effects of IgGFc and/or KT5720 (an inhibitor of PKA) on CGRP release and cAMP levels in DRG neurons isolated from WT mice

To examine whether PKA is involved in IgGFc-induced stimulation of sensory neurons, we analyzed the effect of KT5720 on IgGFc-induced increases in CGRP release and cAMP levels in DRG neurons isolated from WT mice (Fig. 1A, 1B). Pretreatment with KT5720 significantly decreased both CGRP release and cAMP levels in DRG neurons isolated from WT mice (p < 0.01) and it inhibited increase in CGRP release without any effect on cAMP levels in DRG neurons treated with IgGFc (Fig. 1A, 1B).

Effects of IgGFc and/or 4-BPB (an inhibitor of PLA2) on CGRP release from DRG neurons isolated from WT mice

Because activation of PLA2 is critically involved in sensory neuron stimulation (32) and stimulation of FcγRI has been shown to activate PLA2 in human monocytes (33), it is possible that activation of PLA2 is critically involved in the IgGFc-induced stimulation of sensory neurons. To examine this possibility, we analyzed the effect of 4-BPB, an inhibitor of PLA2 (21), on IgGFc-induced increases in both CGRP release and cAMP levels in DRG neurons isolated from WT mice. As shown in Fig. 1, pretreatment with 4-BPB significantly decreased CGRP release and cAMP levels (p < 0.01) and it inhibited IgGFc-induced increases of CGRP release and cAMP levels in DRG neurons isolated from WT mice.

Effects of IgGFc, native IgG, and SB366791 on renal tissue levels of CGRP and IGF-I in WT mice, CGRP⁻/⁻ mice, and denervated WT mice subjected to renal I/R

Both renal tissue levels of CGRP and IGF-I were significantly higher in WT mice subjected to renal I/R than those in WT mice subjected to sham-operation for 6 h after these surgical treatments (p < 0.01) (Fig. 2A, 2B). These levels peaked at 1 h after reperfusion in WT mice that were subjected to renal I/R (Fig. 2A, 2B).
Renal tissue levels of CGRP and IGF-I at 1 h after renal I/R were significantly higher than those at 1 h after sham-operations in WT mice (Fig. 3A, 3B). In contrast, renal tissue IGF-I levels at 1 h after renal I/R were not different from those at 1 h after sham-operation in CGRP–/– mice or denervated WT mice (Fig. 3A, 3B). Administration of IgGFc-enhanced reperfusion-induced increases in renal tissue levels of CGRP and IGF-I at 1 h after renal I/R in WT mice, whereas, it had no effect on these levels in CGRP–/– mice and denervated WT mice (Fig. 3A, 3B). Pretreatment with SB366791, an inhibitor of VR-1 activation (22), inhibited IgGFc-induced increases in renal tissue levels of CGRP and IGF-I in WT mice (Fig. 3). Administration of native IgG did not show any effects on renal tissue levels of CGRP and IGF-I in WT mice subjected to renal I/R (Fig. 3A, 3B).

Effects of IgGFc, native IgG, SB366791, and/or anti–IGF-I Ab on renal tissue levels of caspase-3 and MPO in WT mice, CGRP–/– mice, and denervated WT mice subjected to renal I/R

Renal tissue levels of caspase-3 and MPO were increased at 6 h after renal I/R in WT mice, CGRP–/– mice, and denervated WT mice (Fig. 4A, 4B). Increases in renal tissue levels of caspase-3 and MPO were significantly more marked in CGRP–/– mice and denervated WT mice than in WT mice (Fig. 4). Administration of IgGFc significantly inhibited these increases in WT mice (p < 0.01), but not in CGRP–/– mice or denervated WT mice (Fig. 4). Effects of IgGFc on renal I/R-induced increases in renal tissue levels of caspase-3 and MPO were not observed in WT mice pretreated with SB366791 or in those pretreated with anti–IGF-I Ab (Fig. 4). Administration of native IgG affected none of these variables in WT mice subjected to renal I/R (Fig. 4).

Effect of IgGFc on the number of accumulated neutrophils in kidneys of WT mice, CGRP–/– mice, and denervated WT mice subjected to renal I/R

The number of accumulated neutrophils in kidneys of WT mice, CGRP–/– mice, and denervated WT mice was increased at 24 h after renal I/R (Table I). The increase of the number of accumulated neutrophils in the kidneys was significantly more marked in CGRP–/– mice and denervated WT mice than in WT mice (Table I). Administration of IgGFc significantly inhibited these increases in WT mice (p < 0.01), but not in CGRP–/– mice or denervated WT mice (Table I).

Effects of IgGFc and native IgG on renal tubular apoptosis after renal I/R in WT mice, CGRP–/– mice, and denervated WT mice

Apoptosis as evaluated by TUNEL staining was increased in renal tubular epithelium after renal I/R in WT mice, CGRP–/– mice, and denervated WT mice (Fig. 5). The number of apoptotic cells was apparently higher in CGRP–/– mice and denervated WT mice than in WT mice (Figs. 5, 6). Administration of IgGFc inhibited the renal I/R-induced increase in the number of renal tubular apoptotic cells in WT mice, but not in CGRP–/– mice or denervated WT mice (Figs. 5, 6). In contrast, administration of native IgG did not inhibit the increase in the number of renal tubular apoptotic cells in WT mice subjected to renal I/R (data not shown).

Effects of IgGFc and native IgG, SB366791, and/or anti–IGF-I Ab on the I/R-induced renal dysfunction in WT mice, CGRP–/– mice, and denervated WT mice

Serum levels of BUN and creatinine were increased in WT mice, CGRP–/– mice, and denervated WT mice after renal I/R and such increases were more marked in CGRP–/– mice and denervated WT mice than in WT mice (Fig. 7). Both pre- and posttreatment with SB366791 and anti–IGF-I Ab significantly inhibited these increases in WT mice, CGRP–/– mice, and denervated WT mice (Fig. 7). Both pre- and posttreatment with SB366791 and anti–IGF-I Ab significantly inhibited these increases in WT mice, CGRP–/– mice, and denervated WT mice (Fig. 7).

Table I. Number of accumulated neutrophils in the kidney after reperfusion

<table>
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<tr>
<th>Treatments</th>
<th>(n = 5)</th>
<th>Sham</th>
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<th>I/R and IgGFc</th>
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Number of neutrophils per HPF was counted in kidney samples obtained at 24 h after renal I/R as described in Materials and Methods.

Data are represented as the means ± SD.

*p < 0.01 versus sham.

*p < 0.01 versus I/R.

*p < 0.01 versus WT.
with IgGFc significantly inhibited the renal I/R-induced increases of serum levels of BUN and creatinine in WT mice, but not in CGRP−/− mice or denervated WT mice (Fig. 7). Pretreatment with SB366791 or anti–IGF-I Ab completely inhibited therapeutic effects of IgGFc in WT mice (Fig. 7). Administration of native IgG did not affect renal I/R-induced increases in serum levels of BUN and creatinine in WT mice (Fig. 7).

Effects of IgGFc and native IgG on the I/R-induced renal histological changes in WT mice, CGRP−/− mice, and denervated WT mice

Histological examination of kidneys after renal I/R revealed severe tubular necrosis and obstructing cast in the outer medulla in WT mice, CGRP−/− mice, and denervated WT mice (Fig. 8). Such histological changes were apparently more marked in CGRP−/− mice and denervated WT mice than in WT mice (Fig. 8). The administration of IgGFc reduced these histological changes in WT mice, but not in CGRP−/− mice or denervated WT mice (Fig. 8). In contrast, administration of native IgG did not reduce these histological changes in WT mice (data not shown).

Discussion

In this study, we demonstrated that IgGFc increased CGRP release from DRG neurons isolated from WT mice. Pretreatment with anti-FcγRI Ab reversed the IgGFc-induced increase of CGRP release from DRG neurons isolated from WT mice, suggesting that IgGFc might stimulate sensory neurons by interacting with FcγRI on sensory neurons. We also demonstrated that IgGFc increased cAMP levels in DRG neurons isolated from WT mice. Because cAMP has been shown to sensitize sensory neurons through PKA-dependent phosphorylation of VR-1 (31), it is possible that IgGFc stimulates sensory neurons by increasing intracellular cAMP levels. Consistent with this notion are observations in the current study showing that a PKA inhibitor KT5710 reversed IgGFc-induced increase of CGRP release from DRG neurons.

An inhibitor of PLA2, 4-BBP, reversed the IgGFc-induced increases of CGRP release and cAMP levels in DRG neurons isolated from WT mice. Consistent with these observations, activation of PLA2 has been shown to be involved critically in sensory neuron stimulation (32) and in stimulation of FcγRI in human monocytes (33). However, the mechanism(s) by which arachidonic acid increases CGRP release by increasing cAMP levels is not fully understood. A potent endogenous agonist for sensory neuron stimulation anandamide is synthesized from arachidonic acid in DRG neurons (34) and is capable of increasing cAMP levels in sensory neurons (35). Thus, it is possible that anandamide production is involved in IgGFc-induced sensory neuron stimulation. This possibility should be examined by further experiments in the future.

In contrast to effects of IgGFc, native IgG did not increase CGRP release or cAMP levels in DRG neurons isolated from WT mice. These observations are consistent with a previous report demonstrating that native IgG did not increase substance P release from DRG neurons isolated from mice (5). In this previous report, IgG–Ag complex has been shown to stimulate sensory neurons through activation of FcγRI. These observations suggest that both IgGFc and IgG–Ag complex, but not native IgG, might have the
conformation capable of activating FcγRI on primary sensory neurons. Detailed mechanism(s) for stimulation of sensory neurons via interaction of IgG–Ag complex and IgGFc with FcγRI needs to be elucidated by further experiments.

Administration of IgGFc increased renal tissue levels of CGRP and IGF-I in WT mice, but not in CGRP−/− mice or denervated WT mice, subjected to renal I/R. Pretreatment of WT mice with SB366791 inhibited IgGFc-induced increases in tissue levels of CGRP and IGF-I, thereby decreasing these levels to those observed in sham-operated animals. These observations strongly suggest that SB366791 may inhibit sensory neuron stimulation by IgGFc, and the other endogenous agonists for VR-1 activation. These observations are consistent with our previous report demonstrating that stimulation of sensory neurons with capsaicin increased hepatic tissue levels of CGRP and IGF-I in WT mice, but not in CGRP−/− mice, subjected to hepatic I/R (11). Native IgG did not increase renal tissue levels of CGRP or IGF-I in WT mice subjected to renal I/R. This observation is consistent with results

FIGURE 7. Effects of IgGFc and native IgG on the I/R-induced renal dysfunction (A, B) in WT mice, CGRP−/− mice, and WT/denervation. IgGFc (100 mg/kg) was administered i.v. pre and post. Native IgG (100 mg/kg) was administered i.v. immediately prior to ischemia. SB (500 μg/kg) was administered i.p. 30 min prior to ischemia. Anti–IGF-I ab (50 μg/kg) was administered s.c. 30 min prior to ischemia. Data are expressed as the means ± SD derived from five animal experiments. *p < 0.01 versus sham; †p < 0.01 versus I/R; ‡p < 0.01 versus I/R and IgGFc; §p < 0.01 versus WT. ab; post, 15 min after reperfusion; pre, immediately prior to ischemia; SB, SB366791; WT/denervation, denervated WT mice.

FIGURE 8. Effects of IgGFc and native IgG on the I/R-induced renal histological changes in WT mice, CGRP−/− mice, and denervated WT mice at 24 h after reperfusion. IgGFc (100 mg/kg) was administered i.v. immediately prior to ischemia. H&E staining of kidney was determined in WT mice (A–C), CGRP−/− mice (D–F), and denervated WT mice (G–I) (original magnification ×200). A, D, and G, Sham-operated animals. B, E, and H, I/R animals. C, F, and I, IgGFc-treated I/R animals. Five animals in each group were examined. Typical results are shown.
of in vitro experiments demonstrating that native IgG did not increase CGRP release from DRG neurons isolated from WT mice.

Increases of renal tissue levels of caspase-3 and MPO were inhibited by administration of IgGFc in WT mice subjected to renal I/R. Inhibition of increases of these levels by IgGFc administration was completely reversed by pretreatment with SB366791. Administration of native IgG did not inhibit these increases in WT mice subjected to renal I/R. These observations suggest IgGFc, but not native IgG, may inhibit reperfusion-induced increases in tissue levels of caspase-3 and MPO by stimulating sensory neurons in WT mice. We reported that IgF-I inhibited caspase-3 activation, thereby inhibiting gastric neutrophil accumulation through inhibition of activation of a leukocyte chemoattractant endothelial monocyte activating polypeptide-II in mice subjected to stress (18). Thus, it is likely IgGFc administration might inhibit increases in renal tissue levels of MPO by inhibiting caspase-3 activation through increase of IGF-I production in WT mice subjected to renal I/R.

Activated neutrophils have been shown to be critically involved in the development of I/R-induced acute renal injury by releasing inflammatory mediators, such as neutrophil elastase and reactive oxygen species, are capable of damaging endothelial cells (16). These observations strongly suggest IgGFc may reduce I/R-induced renal dysfunction by inhibiting renal neutrophil accumulation in WT mice.

In addition, renal tubular apoptosis has also been shown to play a critical role in the development of this pathologic condition through induction of tubular cell damage (36). Thus, prevention of renal tubular apoptosis by IgGFc through inhibition of caspase-3 activation might also contribute to reduce both the renal tubular damage and the subsequent renal dysfunction in WT mice subjected to renal I/R. The effect of IgGFc on renal I/R-induced changes observed in WT mice was not observed in CGRP−/− mice or in denervated WT mice, and pretreatment with SB366791 and anti-IGF-I Ab inhibited these therapeutic effects in WT mice subjected to renal I/R. These observations strongly suggest that IgGFc may reduce I/R-induced renal dysfunction by promoting IGF-I production through sensory neuron stimulation.

Taken together, observations in the current study strongly suggest IgGFc, but not native IgG, may be capable of stimulating sensory neurons, thereby increasing IGF-I production, which may reduce I/R-induced renal injury by inhibiting neutrophil accumulation and renal tubular apoptosis in mice.

Observations in the current study raised the possibility that IgGFc has the potential for the treatment of severe sepsis in which inflammation and renal tubular apoptosis in mice.

reduce I/R-induced renal injury by inhibiting neutrophil accumulation through inhibition of a leukocyte chemoattractant endothelial monocyte activating polypeptide-II in mice subjected to stress (18). Thus, it is likely IgGFc administration might inhibit increases in renal tissue levels of MPO by inhibiting caspase-3 activation through increase of IGF-I production in WT mice subjected to renal I/R.

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Observations in the current study raised the possibility that IgGFc has the potential for the treatment of severe sepsis in which activated neutrophils play a critical role in the development of organ failures (37).

Disclosures
The authors have no financial conflicts of interest.

References


Letter of Retraction

We wish to retract the article titled “Stimulation of FcγRI on Primary Sensory Neurons Increases Insulin-Like Growth Factor-I Production, Thereby Reducing Reperfusion-Induced Renal Injury in Mice” by Naoaki Harada, Juan Zhao, Hiroki Kurihara, Naomi Nakagata, and Kenji Okajima, The Journal of Immunology, 2010, 185: 1303–1310.

Panels C–H of Fig. 5 were incorrectly inserted by the first author, Dr. Naoki Harada. Dr. Harada made this figure for practice for a presentation at an International Congress. He drew data from another experiment in which the same procedure was done. At the time, he did not have correct data because he had not finished experiments to produce genuine data for the figure. Although he went on to finish experiments to get genuine data, he forgot to replace the figure with the correct one before the paper was submitted to The Journal of Immunology. Although the submission of the incorrect figure was unintentional, we hereby withdraw our article.

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