Expression of retinoic acid-inducible gene-I in lupus nephritis

Sir,

Retinoic acid-inducible gene-I (RIG-I) is a member of the DExH-box family of proteins, which is involved in inflammatory reactions related to RNA metabolisms, although the bioactivities of RIG-I still remain to be clarified in detail [1]. Previous studies have reported that expression of RIG-I is induced in various inflammatory diseases, such as viral infections, leukaemia and bladder carcinoma [1]. However, there are no reports on the expression of RIG-I in the kidneys of patients with lupus nephritis. In the present study, we examined the expression of RIG-I in kidney tissue specimens obtained from cases of human lupus nephritis, and evaluated the correlation between its expression and the histological activity of the renal disease in these patients.

From January 2000 to August 2006, lupus nephritis was diagnosed in 15 children and adolescents at Hirosaki University Hospital. All met the International Society of Nephrology/Renal Pathology Society (ISN/RPS) criteria for the histological diagnosis of lupus nephritis. Tissue cylinders obtained by renal biopsy were divided into three portions and processed for routine light-microscopic, electron-microscopic and immunofluorescence examinations. After the renal tissue samples for the immunofluorescence study were embedded in optimal cutting temperature (OCT), routine studies to determine the deposition of IgG, IgA, IgM, C3 and C1q were performed, and the samples were stored at −30°C until further use. Of these OCT-embedded kidney tissue specimens, 10 specimens obtained from eight patients, in good condition, were selected for this study. Repeat renal biopsy was performed in two of the eight patients (cases 3 and 6) who exhibited changes of proliferative lupus nephritis at presentation. Eight kidney tissue specimens obtained from patients with minimal-change (MC) disease were used as controls.

Each of the kidney tissue specimens from the patients with lupus nephritis was examined by light microscopy in a blinded fashion by one of the examiners and the histological findings were scored. The activity index was evaluated semi-quantitatively using the scoring system described by Austin et al. [2].

The OCT-embedded tissue specimens were cut into 5μm-thick sections in a cryostat, briefly fixed in cold acetone and then air-dried; the slides were then washed in PBS immediately before the immunohistochemical procedure. After blocking by incubating with 1% goat serum, the slides were incubated with anti-RIG-I antibody (1:1000), as described by Imaizumi et al. [1]. The samples were then incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (Sigma, Saint Louis, USA).

According to ISN/RPS criteria for light-microscopic histological classification, two patients (cases 2 and 8) were classified as class II nephritis, four (cases 1, 3, 4 and 6) as class IV nephritis, and two (cases 5 and 7) as class V nephritis. The mean activity index was 6.3, and two patients (cases 3 and 6) had a very high activity index of more than 10 (Table 1).

Significant histological improvement was observed at the second renal biopsy in both cases 3 and 6. According to the ISN/RPS criteria, case 3 was classified as class II and case 6 as class III. Indeed, the activity index was also significantly decreased in both the cases (to three in case 3 and two in case 6).

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at biopsy</th>
<th>Sex</th>
<th>Number of glomeruli</th>
<th>Histological grade</th>
<th>ISN/RPS criteria</th>
<th>Activity index</th>
<th>Immunofluorescence examination RIG-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>12</td>
<td>36</td>
<td>Class IV</td>
<td>Class IV</td>
<td>3</td>
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<td>2</td>
<td>M</td>
<td>15</td>
<td>15</td>
<td>Class IV</td>
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</tr>
<tr>
<td>3</td>
<td>M</td>
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<td>28</td>
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<td>5</td>
<td>F</td>
<td>26</td>
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<td>Class V</td>
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<td>8</td>
<td>M</td>
<td>10</td>
<td>11</td>
<td>Class III</td>
<td>Class III</td>
<td>2</td>
<td>++</td>
</tr>
</tbody>
</table>

Table 1. Summary of the pathological and immunohistochemical findings in the eight patients with lupus nephritis.

1+, slightly positive; 2+, positive; 3+, markedly positive; examined by immunofluorescence.
and seven in case 6). None of the patients with MC disease showed any more than minor glomerular abnormalities on light-microscopic examination.

Glomerular immunoreactivity for RIG-I was detectable in all the cases of lupus nephritis and an intense granular pattern of immunofluorescence was observed in a mesangial area and capillary loop distribution. Immunoreactivity of variable intensity was also present in the interstitial lesions from the patients with lupus nephritis. The intensity of the immunostaining correlated well with the renal histological activity index, but not with the findings of routine immunofluorescence examination. Especially, cases with high fluorescence intensity signals for RIG-I expression showed severe leucocyte exudation in the glomeruli on light-microscopic examination (Table 1). In cases 3 and 6 with a high index of histological activity, at the first biopsy in particular, a significantly increased intensity of immunostaining for RIG-I was observed (Figure 1). Furthermore, the immunostaining intensity was decreased at the second biopsy in both the cases. In the cases with MC disease, immunofluorescence staining was either absent or only trace-like in scattered areas of the glomeruli.

It has been reported that Th1-derived cytokines play an important role in the progression of lupus-associated renal injury [3]. Interferon γ (IFN-γ) has been shown to induce the expression of various factors, including adhesion molecules, chemokines, growth factors and enzymes in both animal models and human lupus nephritis, and is believed to play an important role in the inflammatory and immune processes involved in the pathogenesis of lupus nephritis [3]. Patole et al. [3] reported that the expression of toll-like receptors (TLRs) was up-regulated in lupus-like immune complex glomerulonephritis of MRL-Fas(lpr) mice, and the regulation of TLRs was involved in infection-associated exacerbation. In contrast, it has been reported that up-regulation of interleukin-4, a Th2-derived cytokine, may represent the T cell dysfunction involved in MC disease [4]. In the present study, our results revealed strong immunostaining for the RIG-I protein in the kidney specimens obtained from the cases with lupus nephritis, but negative or only very weak staining in the specimens obtained from the cases with MC disease. Imaizumi et al. [1] reported that RIG-I expression may mainly contribute to the Th1 type reaction, because its expression is induced in human endothelial cells stimulated by IFN-γ. Also, Paladino et al. [5] reported that TLRs and RIG-I would serve an important role during the type I IFN-dependent antiviral response in epithelial and fibroblast cell layers. Considering the reports in the literature and our own results, we suggest that the Th1 type reaction may play an important role in the expression of RIG-I in the human kidney. To the best of our knowledge, this is the first report showing the expression of

Fig. 1. (A) First renal biopsy specimen in case 3 with lupus nephritis. Significant increase in the immunostaining intensity for RIG-I was observed. The histological picture by light microscopy was classified as class IV according to the ISN/RPS criteria and the activity index was 14. (B) Second renal biopsy in case 3. The histological picture was classified as class II and the activity index was 3. The immunostaining intensity for RIG-I was decreased compared with that seen in the first renal biopsy. (C) Renal biopsy specimen from a patient with MC disease. Immunofluorescence staining for RIG-I was absent.
RIG-I in diseased human kidneys by an immunohistochemical technique.

In this study, it appeared that the amount of immunohistochemically detectable RIG-I was related to the severity of the glomerular lesions, such as that of leucocyte exudation into the glomeruli, in the specimens obtained from cases with active lupus nephritis. Two cases, with severe histological changes and a very high histological activity index, showed significantly more intense immunostaining for RIG-I. Furthermore, the staining intensity decreased in parallel with the improvement of the histological activity index at the second biopsy. These observations suggest that the expression of RIG-I in lupus nephritis could be useful as a parameter for reflecting the histological activity and severity of the renal disease.

In conclusion, we demonstrated that RIG-I expression occurs at levels detectable by indirect immunofluorescence examination, and that the intensity of its expression is correlated with the histological activity index in cases of lupus nephritis. The mechanisms by which RIG-I mediates the inflammatory and immunological processes involved in lupus nephritis still remain to be determined, and must be addressed in future studies.

Conflict of interest statement. None declared.

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5. Paladino P, Cummings DT, Noyce RS, Mossman KL. The IFN-independent response to virus particle entry provides a first line of antiviral defense that is independent of TLRs and retinoic acid-inducible gene I. J Immunol 2006; 177: 8008–8016

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Cinacalcet modifies the pH of solutions in vitro: possible implications for gastro-intestinal side effects in vivo

Sir,

The US-based Federal Drug Agency (FDA) and the European Medicines Agency have approved the use of cinacalcet HCl for the treatment of secondary hyperparathyroidism in patients on dialysis, and in those with parathyroid carcinoma [1]. Cinacalcet is effective and, overall, well tolerated [2]. However, in clinical trials, it has been observed that certain patients on cinacalcet have episodes of hypocalcaemia (1.4% of patients), nausea (~31%) and vomiting (~27%), much more than in patients receiving a placebo [3,4]. Gastrointestinal intolerance, with vomiting in particular, has been found to be dose-related, and is the most common reason for drug discontinuation. Moreover, cinacalcet therapy can exacerbate oesophago-gastro-duodenal disease in ESRD patients, gastric fluid pH ranging from acid excess to achlorhydria. The aim of our study was to clarify whether the in vitro dissolution of commercially available cinacalcet causes medium pH variations in solutions with different basal proton concentrations, simulating gastric, duodenal and intestinal pH, respectively.

Cinacalcet 60 mg (AMGEN Europe B.V.) was dissolved following the US dissolution II paddle method at a rotation speed of 50 r.p.m. in 900 ml of dissolution medium at a stable temperature of 37 ± 0.01°C, maintained by a Haake cryostat.

pH variations were determined by a pH-meter in solutions at different pH, corresponding to the fluids of different gastro-intestinal segments: 1.90, 5.90, 7.51 [5]. The dissolution profile was obtained from the change of the area under the spectral profile observed using spectrophotometric UV measurements in the 230–320 nm range, while the profile of the change of pH was obtained by introducing a pH-meter into the solutions. Variations in pH were graphically reproduced using Prism Statistical software (version 4.00; Graphpad, San Diego, CA, USA). Each value represents the average of acquisition replicates of nine measurements.

Dissolution of 60 mg of cinacalcet in an acid pH (1.90) medium did not modify the acidity of the solution, whereas in an alkaline solution, the drug induced a rapid increase in acidity (from pH 7.50 to pH 6.70). Finally, the change in proton concentration of the solution at pH 5.90, simulating the duodenal environment, is of particular interest, since it changed towards significant acidity (5.30) Fig.1. Notably, pharmacokinetic findings showed that the absorption of cinacalcet is dominantly duodenal. The observed pH changes in vitro would suggest that, in vivo, orally ingested cinacalcet might rapidly transform alkaline gastrointestinal fluids into acid fluids. This could be of clinical relevance in ESRD patients who ingest the drug.

The acidifying action of cinacalcet derives from its chemical composition, a phenylalkylamine compound with a fluorine radical. We used trehalose in order to modulate the acidifying effect of cinacalcet. This disaccharide is used with increasing frequency to stabilize pharmaceutical products, and it has a significant effect on H-binding structures [6]. The addition of trehalose together with cinacalcet 60 mg to the medium at a pH of 5.9 allowed the pH value to remain stable, while the drug dissolved rapidly and completely.

In addition to this in vitro experiment, we performed 24-h oesophago-gastrointestinal pH measurement in a 56-year-old Helicobacter Pylori-negative male haemodialysis patient, who was receiving a proton pump inhibitor. He was given cinacalcet because of secondary hyperparathyroidism. After administration of the drug, a 5 cm electrode was positioned at the oesophago-gastro-duodenal junction and another in the stomach, to evaluate pH changes. The patient took three meals (breakfast, lunch and dinner). He was in the upright position for 14 h 30 min and in a supine position for 9 h 30 min. Both after breakfast and lunch, the patient