Role of Intercellular Adhesion Molecule-1, Lymphocyte Function-associated Antigen-1, and Macrophages in ddY Mouse Nephropathy

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ABSTRACT

ddY mouse nephropathy is an animal model of human IgA nephropathy that is characterized by spontaneous IgA deposition in the glomerular mesangium, mesangial cell proliferation, and matrix expansion. We investigated the involvement of intercellular adhesion molecule-1, lymphocyte function-associated antigen-1, and macrophages in the pathogenesis of ddY mouse nephropathy. Five mice each underwent urinalysis, light microscopic examination of the kidneys, immunofluorescent detection of immunoglobulins and complement, and immunohistochemical examination for intercellular adhesion molecule-1, lymphocyte function-associated antigen-1, and infiltrating macrophages at 5, 10, 20, 30, 40, 50, 60, and 70 weeks of age. Albuminuria was observed from the age of 20 weeks and all mice showed albuminuria by 70 weeks. Histological glomerular damage was significantly related to the appearance of albuminuria (p<0.01). In the glomeruli, positivity for intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1, as well as the number of infiltrating macrophages, were significantly increased in mice with nephropathy compared to pre-nephropathy mice (p<0.01). These results suggest that intercellular adhesion molecule-1, lymphocyte function-associated antigen-1, and infiltrating macrophages are involved in the progression of histological damage in ddY mouse nephropathy.

Key words: ddY mouse, Intercellular adhesion molecule-1, Lymphocyte function-associated antigen-1, Macrophages

MATERIALS AND METHODS

Urinalysis and preparation of specimens

Forty ddY mice were used for this study. Albuminuria and hematuria were detected using dip sticks (Ames urine test paper, Lifestics; Bayar-Sankyo Co. Ltd., Tokyo, Japan). Five mice each, aged 5, 10, 20, 30, 40, 50, 60, and 70 weeks, were sacrificed under ethyl ether anesthesia. Based on the results of a previous investigation and our preliminary study, the mice were divided into 2 groups: mice without albuminuria formed the pre-nephropathy group (group I) and mice with albuminuria formed the nephropathy group (group II). There were 17 mice in group I and 23 mice in group II.
After each mouse was killed, the right kidney was immediately excised and specimens were prepared for histological, immunofluorescence, and immunohistochemical studies. Specimens for histological and immunohistochemical examination were fixed in 10% neutral buffered formalin for 24 hours and then embedded in paraffin. Specimens for immunofluorescence studies were immediately embedded in OCT compound (Tissue-Tek, Miles Inc., Elkhart, USA) and subsequently snap-frozen without fixation and stored at -80 °C until use.

**Histology**
Specimens embedded in paraffin were cut into 3-μm sections and stained with periodic acid-Schiff (PAS) stain. The extent of mesangial cell proliferation and matrix expansion was observed by light microscopy, and glomerular histological damage was assessed as follows: a score from 0 to 3 points was assigned for mesangial cell proliferation and matrix expansion in each glomerulus (Fig. 1), and the number of points was summed for 30 glomeruli to give a histological damage score.

**Immunofluorescence**
Unfixed specimens stored at -80 °C were cut into 4-μm sections. Then the sections were placed on poly-L-lysine-coated glass slides, air-dried at room temperature for 60 min, and washed with phosphate-buffered saline (PBS, pH 7.4). Next, the sections were stained using fluorescein isothiocyanate-conjugated rabbit anti-mouse polyclonal antibodies for IgA, IgG, and IgM (Zymed Laboratories Inc., San Francisco, USA), as well as an antibody for C3 (Medical and Biological Laboratories, Nagoya, Japan).

**Immunohistochemistry**
Specimens embedded in paraffin were cut into 3-μm sections and placed on poly-L-lysine-coated glass slides. Endogenous peroxidase activity was blocked by treatment with 0.15% hydrogen peroxide in methanol for 15 min, after which the sections were washed with PBS (pH 7.6) for 10 min, incubated at 37 °C with trypsin for 30 min, and washed again with PBS for 10 min. Then the sections were incubated for 12 hours at 4 °C in a moist chamber with monoclonal rat anti-mouse ICAM-1 and LFA-1 antibodies (Genzyme, Cambridge, USA), as well as a monoclonal rat anti-mouse macrophage antibody (Biomedicals AG, Augst, Switzerland). After being thoroughly washed with PBS, the sections were stained using an ABC kit (Vectastain, Vector Laboratories, Inc., Burlingame, USA). The sections were subsequently reacted with 3,3'-diaminobenzidine tetrahydrochloride (Wako Pure Chemicals Ltd., Osaka, Japan), and the nuclei were counterstained with methyl green.

ICAM-1-positive glomeruli were scored according to the semiquantitative method of Bertoluci et al.[8]. Briefly, the immunohistochemical reaction was graded as 0 for no staining, 1 for staining of 25% of the glomerular tufts, 2 for 50% staining, 3 for 75% staining, or 4 for more than 75% staining. A score was then calculated using data from 30 glomeruli in each mouse as follows: Score = ΣFi(i), where Fi is the percentage of glomeruli in the mouse with a given score of i. LFA-1-positive cells and anti-macrophages antibody-positive cells were also counted in 30 glomeruli. All slides were evaluated by two independent investigators and differences in assessment were resolved by consensus.

Statistical analysis was performed using regression analysis for correlation coefficients and the unpaired Student's t-test for significance. Probability values of less than 0.05 were considered to indicate significance.

**RESULTS**

**Urinalysis**
No albuminuria was observed in any of the mice at the ages of 5 and 10 weeks, but was detected from 20 weeks onwards. Three out of five mice showed albuminuria at the age of 30 weeks, and 4 out of 5 mice showed from 40 to 60 weeks. At the age of 70 weeks, all five mice had albuminuria (ranging from 30 to 100 mg/dl). No hematuria was observed in any of the mice.

<table>
<thead>
<tr>
<th>Table 1. Comparison of various parameters in groups I and II</th>
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<tr>
<td><strong>proteinuria</strong></td>
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<td>histological damage score</td>
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<tr>
<td>ICAM-1 score</td>
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<tr>
<td>No. of LFA-1-positive cells</td>
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<td>No. of macrophages</td>
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Results are expressed as the mean ± standard error. Probability values are for comparisons between the two groups.
Table 2. Relationships among ICAM-1, LFA-1, and macrophages

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<th>n=40</th>
<th>significance</th>
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<tr>
<td>ICAM-1 score versus No. of macrophages</td>
<td>r=0.631</td>
<td>p&lt;0.01</td>
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<tr>
<td>No. of LFA-1-positive cells versus No. of macrophages</td>
<td>r=0.626</td>
<td>p&lt;0.01</td>
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<tr>
<td>ICAM-1 score versus No. of LFA-1-positive cells</td>
<td>r=0.782</td>
<td>p&lt;0.01</td>
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Regression analysis was used to calculate correlation coefficients (r values).

Table 3. Relationships among histological damage, adhesion molecules, and macrophages

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<th>n=40</th>
<th>significance</th>
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<tr>
<td>histological damage versus ICAM-1 score</td>
<td>r=0.444</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>histological damage versus No. of LFA-1-positive cells</td>
<td>r=0.354</td>
<td>p&lt;0.05</td>
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<tr>
<td>histological damage versus No. of macrophages</td>
<td>r=0.374</td>
<td>p&lt;0.05</td>
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Regression analysis was used to calculate correlation coefficients (r values).

Fig. 1. Photomicrographs showing histological scoring of glomerular damage.
(A) No abnormal findings = 0 points (PAS, × 200). (B) Mild matrix expansion and cell proliferation = 1 point (PAS, × 200). (C) Moderate matrix expansion and cell proliferation = 2 points (PAS, × 200). (D) Severe matrix expansion and cell proliferation = 3 points (PAS, × 200).

Fig. 2. Direct immunofluorescence detection of immunoglobulins and complement in the glomeruli (× 200). The number of immunofluorescent-positive glomeruli increased with age. After 30 weeks, all the glomeruli were positive for IgA (A), IgG (B), IgM (C), and C3 (D).

Fig. 3. Immunohistochemical localization of ICAM-1 in the glomerulus (× 100). This ICAM-1-positive glomerulus was scored as 3 points.

Fig. 4. Immunohistochemical localization of LFA-1 in the glomerulus (× 100). Two LFA-1-positive cells can be seen in the glomerulus.

Fig. 5. Immunohistochemical localization of macrophages in the glomerulus (× 100). Two macrophage monoclonal antibody-labeled cells can be seen in the glomerulus.
Histological findings

No histological changes of the kidney were observed in any of the mice aged 5 and 10 weeks. From 20 weeks onwards, the cellularity of the mesangial region showed an increase. Cell proliferation was accompanied by an increase of mesangial matrix, and mesangial sclerosis was found from 30 weeks onwards in severe cases. However, the interstitium showed few changes even though glomerular damage was severe in the mice with nephropathy.

In group II (nephropathy mice), the histological damage score was significantly higher than in group I (pre-nephropathy mice) (p<0.01, Table 1).

Immunohistochemical findings

Deposits of IgG and IgM in the mesangial region were observed from 10 weeks onwards, while deposits of IgA and C3 were observed from 20 weeks (Fig. 2). After the age of 30 weeks, IgA deposition was most prominent. There was no relationship between the intensity of immunofluorescence for any of the markers studied and albuminuria or histological damage.

Immunofluorescence findings

ICAM-1 expression was observed on the luminal surface of the glomerular endothelium, in the mesangium, in the parietal epithelium of Bowman’s capsule, and in the tubular epithelium (Fig. 3). Cells labeled by the LFA-1 monoclonal antibody (Fig. 4) and some infiltrating macrophages (Fig. 5) were found in the same glomeruli. ICAM-1 showed progressive up-regulation, and LFA-1-positive cells and infiltrating macrophages also increased significantly with the exacerbation of histological damage. In mice with nephropathy (group II), ICAM-1 and LFA-1 positivity as well as infiltrating macrophages were significantly increased compared to the pre-nephropathy mice (group I) (Table 1). As shown in Table 2, there were significant positive correlations among the ICAM-1 score, LFA-1-positive cells, and the number of infiltrating macrophages. The relationships among histological damage, adhesion molecules, and infiltrating macrophages are shown in Table 3. The level of positivity for ICAM-1 and LFA-1 as well as the number of infiltrating macrophages were significantly correlated with exacerbation of histological damage. A few cells labeled by the LFA-1 or macrophage monoclonal antibodies were also found in the tubular interstitium.

DISCUSSION

IgA nephropathy features IgA deposition in the mesangial region, mesangial cell proliferation, and mesangial matrix expansion, but the pathogenesis of this disease still remains unclear. The present study examined the role of adhesion molecules and macrophages in the pathogenesis of ddY mouse nephropathy. The nephropathy that occurs in ddY mice is not strictly equivalent to human IgA nephropathy, because these mice do not have hematuria and there are some other differences. However, ddY mice show albuminuria, mesangial IgA deposition, mesangial cell proliferation, and matrix expansion, thus providing a reasonable approximation to human IgA nephropathy.

In this study, we divided the mice into two groups depending on the level of albuminuria, because this is one of the main parameters used clinically for the detection of nephropathy. Histological damage was assessed from the extent of mesangial cell proliferation and matrix expansion in 30 PAS-stained glomeruli from each mouse, with the results being expressed as a histological damage score. The relationship between histological damage and renal function was not investigated because we did not determine the glomerular filtration rate or creatinine clearance. However, the histological damage score was significantly related to the appearance of albuminuria (p<0.01), which should reflect the severity of nephropathy.

Macrophages have been shown to mediate glomerular damage in several of the glomerulonephritides. Their functions range from degradation of biological debris to promotion of inflammation. In the present study, a positive correlation was noted between histological damage and the infiltration of macrophages (p<0.05). This suggests that macrophages are not only involved in acute glomerulonephritis, but also in the chronic glomerular changes of ddY mouse nephropathy. It is known that adhesion molecules are important in mediating the role of macrophages in the inflammatory response. Expression of ICAM-1 has been reported in various types of glomerulonephritis. As far as we know, however, this is the first study to show that the expression of LFA-1 increases significantly with the exacerbation of histological damage in ddY mouse nephropathy. Macrophages must adhere to the vascular endothelium before migrating from the circulation to sites of inflammation. The interaction of LFA-1 with its counter-receptor, ICAM-1 (expressed by the glomerular endothelium and mesangium), mediates macrophage migration from the circulation to the glomeruli. A high level of expression of ICAM-1 is seen at sites of inflammation, and ICAM-1 expression on endothelial cells is enhanced by proinflammatory cytokines such as tumor necrosis factor, interleukin-1, and interferon-γ. In addition, glomerular damage can be reduced by an anti-ICAM-1 and LFA-1 antibody, indicating that ICAM-1 and LFA-1 play an important role in mediating macrophage-endothelial cell interactions in the inflammatory process of nephropathy.
Adherent and migrating macrophages express LFA-1, which binds with ICAM-1 on endothelial cells as well as other leukocytes(3,17,18). Activated macrophages are recruited to sites of inflammation where these cells release several cytokines and other biological mediators (proteolytic enzymes, eicosanoids, reactive oxygen species, etc.) that could be involved in the inflammatory response and/or the progression of ddY mouse nephropathy.

Tomino et al(20) have reported that IgA deposition in the glomeruli is not directly related to increased glomerular expression of ICAM-1. However, ICAM-1, LFA-1 and macrophages are thought to be involved in several types of glomerulonephritis, and up-regulation of adhesion molecule expression may promote infiltration of macrophages into the glomeruli, which may be involved in the exacerbation of glomerular damage. In this study, only a few LFA-1-positive cells and macrophages were found in the interstitium, and there was little interstitial damage. Accordingly, we only evaluated the glomerular localization and the relationship to histological damage of ICAM-1, LFA-1, and macrophages.

Age-associated changes of the glomeruli have been reported to occur in mice. However, Yumura et al(21) found that C57BL/6 mice, which do not develop any specific diseases in the course of senescence, did not show sclerosis of the mesangial matrix up to the age of 24 months (104 weeks). In the present study, histological damage was found from 20 weeks onwards in group II, and mesangial sclerosis was also found in some mice after 30 weeks. Thus, there would seem to be little influence of aging on the nephropathy detected in our study.

In conclusion, ICAM-1, LFA-1, and infiltrating macrophages appear to be involved in the exacerbation of glomerular damage in ddY mouse nephropathy.

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