Intercellular adhesion molecule-1 mediated interactions and leucocyte infiltration in IgA nephropathy

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Abstract

Background. Mononuclear leucocytes have a role in IgA nephropathy (IgAN). Renal leucocyte recruitment is mediated by adhesive interactions between leucocytes and their ligands on renal cells.

Methods. We have assessed interstitial and glomerular leucocytes by avidin-biotin-peroxidase with monoclonal antibodies (MA) against leucocytes (CD45), β₂-integrin (CD18), monocyte-macrophages (CD14), T (CD3) and T-cell subsets (CD4, CD8), and intercellular adhesion molecule-1 (ICAM-1) (CD54), and analysed their relation with the abnormal expression of ICAM-1 on proximal tubule epithelium in sequential renal sections from 48 patients with IgAN stratified according to the severity of the interstitial cellular infiltration observed by light microscopy.

Results. In IgAN without (n=15) and with (n=7) interstitial cellular infiltration of 1 +, ICAM-1 expression on vascular endothelium was unchanged with respect to that observed in the normal kidney; the proximal tubule epithelium was negative for this stain. In IgAN with interstitial cellular infiltration of 2 + (n=10), 3 + (n=11), and 4 + (n=5), ICAM-1 stain was observed on the proximal tubule epithelium, the median value of its quantitative expression being 0.3, 0.1, and 0.2 (P=0.0008), respectively. The tubular ICAM-1+ stain was significantly associated with the interstitial leucocytes identified by MA, and correlated with CD45+ (r=0.59, P=0.02), CD14+ (r=0.54, P<0.02), and CD3+ (r=0.51, P=0.02) interstitial leucocytes in IgAN with interstitial cellular infiltration. Interstitial ICAM-1+ and CD18+ leucocytes were correlated (r=0.56, P<0.001). Correlation was found between the quantitative tubular expression of ICAM-1+ and the number of CD45+ (r=0.98, P<0.0001), CD3+ (r=0.48, P=0.02), and CD8+ (r=0.76, P<0.02) glomerular leucocytes.

Conclusions. Our results suggest that tubular and interstitial ICAM-1+ cells may participate in adhesive interactions with interstitial leucocytes. Intestinal T-cells and macrophages as well as glomerular T-cells bearing predominantly CD8+ phenotype could play a role in the induction of the tubular expression of ICAM-1 in IgAN.

Key words: IgA nephropathy; interstitial and glomerular leucocyte infiltration; intercellular adhesion molecule-1

Introduction

Primary IgA nephropathy (IgAN) is the most widespread glomerulonephritis (GN) in the developed world [1]. The disease is characterized by mesangial IgA deposition, often in conjunction with C3 and IgG and/or IgM, and it appears with a highly variable degree of glomerular hypercellularity [2]. Peripheral [3] and histological studies have documented that cell-mediated immunity is involved, particularly at the intratubular level [4–6], in IgAN. Renal recruitment of immunocompetent leucocytes is mediated by interactions between adhesion molecules expressed on leucocytes and their ligands on cells of the kidney. A fundamental ligand in leucocyte trafficking is the intercellular adhesion molecule-1 (ICAM-1) belonging to the immunoglobulin superfamily, which is upregulated by interleukin-1 (IL-1), tumour necrosis factor (TNF)-α and interferon-γ [7]. ICAM-1 expression is present on normal renal vascular endothelium [8,9], and induction is reported on mesangium [10,11], parietal, and tubular epithelium [12,13] in diverse types of GN. In IgAN, the expression of ICAM-1 remains unclear [8,12,14,15]. In order to examine potential ICAM-1-mediated leucocyte interactions in IgAN, we have characterized and analysed the correlation between the composition of interstitial and glomerular infiltration of leucocytes using monoclonal antibodies (MA), including CD18 MA against the common β-chain of their counter-ligands leucocyte-function-associated antigen-1 (LFA-1) and Mac-1 [7], and the tubular and
interstitial expression of ICAM-1 in sequential histological sections of the same renal biopsy.

Materials and methods

Tissue

Diagnostic renal biopsies from 48 patients with primary IgAN were studied. None had clinical or biochemical evidence of liver disease, Henoch-Schönlein purpura, or systemic lupus erythematosus. Ten normal renal tissues were used as control. Biopsy specimens processed for routine light and immunofluorescence microscopy were classified according to glomerular pathology and degree of the interstitial cellular infiltration seen by light microscopy (Table 1).

Immunohistochemistry

Kidney sections were tested by avidin-biotin-peroxidase [13] using MA against leucocyte common antigen (CD45, 72-5-D3), β1-integrin (CD18, 65-5-A5), monocyte-macrophages (CD14, Cris-6), helper/inducer T cells (CD4, Leu-3a), cytotoxic/suppressor T cells (CD8, Leu-2a), and intercellular adhesion molecule-1 (ICAM-1) (CD54, RM-3-A5). This last MA was applied after preincubation with egg albumin to block renal biotin. Tonsil tissue and slides from each specimen incubated with ascites fluid of myeloma NS1 or PBS instead of MA were used as control. Biopsy specimens processed for routine light and immunofluorescence microscopy were classified according to glomerular pathology and degree of the interstitial cellular infiltration seen by light microscopy (Table 1).

Evaluation

A Leitz microscope at magnification ×400 with an ocular grid (0.07 mm²) was used. Interstitial leucocytes identified by any of the above MA were counted in 10–20 adjacent fields adjusted to avoid glomeruli and major vessels in an area of at least 0.2 mm² on sequential renal sections. The number of positive leucocytes per mm² of interstitium was calculated. Glomerular leucocytes identified by any of the above MA except CD54 were counted in all glomeruli. The number of positive leucocytes per mm² of glomerular area was calculated. Tubular epithelial positive cells for CD54 were noted as present or absent. CD54 staining on proximal tubule epithelium was counted in 10–20 tubular cross-sections. Square millimetre of CD54+ proximal tubule epithelium per mm² of tubular area ×10 was calculated.

Statistics

Data were analysed with SPSS. Win 6.1.3 programs using non-parametric Mann-Whitney U-test and Spearman’s correlation coefficient. Significant level was considered for P<0.05.

Results

ICAM-1 antigens and leucocytes in normal kidney

ICAM-1 (CD54) antigens were strongly expressed on vascular endothelium. Tubular epithelium was negative for this stain. Occasional stain for CD45, CD18, and CD14 MA, but no stain for CD3, CD4, or CD8 MA within interstitium or glomerulus were seen in normal renal tissue.

ICAM-1 antigens and interstitial leucocytes in IgAN

A common pattern of ICAM-1 antigens was unchanged with respect to that observed in the normal kidney in IgAN without interstitial cellular infiltration and with interstitial cellular infiltration of + (Figure 1a). In IgAN with interstitial cellular infiltration ≥2+ moreover, ICAM-1 expression was seen on proximal tubule epithelium (Figure 1b), the median value of ICAM-1+ proximal tubule epithelium was 0.3 (range 0.1–0.4), 0.1 (range 0–0.2), and 0.2 (range 0–0.3) (P=0.0008) in the biopsies with 2+, 3+, and 4+ of interstitial cellular infiltration, respectively. The biopsies with ICAM-1+ stain on proximal tubule epithelium showed a number of interstitial leucocytes identified by any of the above MA significantly greater than the biopsies without ICAM-1 stain on proximal tubule epithelium; the correlation between the quantitative ICAM-1 tubular staining and the number of leucocytes being significant (Table 2). The biopsies with interstitial cellular infiltration (n=33) confirmed the correlation between the ICAM-1+ tubular stain and the number of CD45+ (r=0.59, P=0.02), CD14+ (r=0.54, P=0.02) and CD3+ (r=0.51, P=0.02) interstitial leucocytes. A correlation (P<0.001) was found between the number of CD54+ leucocytes and CD45+ (r=0.89), CD18+ (Figure 2) (r=0.56), CD14+ (r=0.66), CD3+ (r=0.79), CD4+ (r=0.85), and CD8+ (r=0.81) leucocytes infiltrating the interstitium.

ICAM-1 antigens and glomerular leucocytes in IgAN

The biopsies with ICAM-1+ stain on proximal tubule epithelium showed a significantly greater number of CD45+, CD3+, and CD8+ glomerular leucocytes than the biopsies without ICAM-1 stain on proximal tubule epithelium, the correlation (r=0.98, P<0.0001; r=0.48, P=0.02; r=0.76, P<0.02, respectively) being significant (Table 3).

<table>
<thead>
<tr>
<th>Glomerular pathology</th>
<th>Interstitial cellular infiltration</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor</td>
<td>−</td>
<td>13</td>
</tr>
<tr>
<td>Focal GN</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Diffuse GN</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Sclerosis</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>10</td>
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<tr>
<td></td>
<td>++</td>
<td>11</td>
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<td>5</td>
</tr>
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<td>++++</td>
<td>48</td>
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</tbody>
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*Minor glomerular abnormality when <20% of glomeruli showed proliferative lesions. Focal GN when 20–80% of the glomeruli showed proliferative lesions. Diffuse proliferative GN when 80% of the glomeruli showed proliferative lesions. Advanced sclerosis when >80% of the glomeruli showed predominant sclerotic lesions rather than proliferation.

+Interstitial cellular infiltration negative, + when cells were sparsely scattered, ++ when less than three infiltrate foci were detected, +++ when the number of foci was more than three, ++++ when the whole tissue was infiltrated.

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Table 1. Glomerular pathology and degree of interstitial cellular infiltration seen by light microscopy in IgA nephropathy
Fig. 1. (a) ICAM-1 (CD54) antigens identified on the arterial endothelium and peritubular capillaries. The tubular epithelium is negative for this stain in a biopsy with IgA nephropathy classified as focal GN. (b) ICAM-1 (CD54) antigens identified on the proximal tubule epithelium in a biopsy with IgA nephropathy classified as diffuse proliferative GN. Avidin-biotin-peroxidase. (× 250).

Discussion

In IgAN, the tubulointerstitial expression of ICAM-1 remains discordant (8,12,14,15) due in part, to examinations performed in different phases of the disease. This histological study of IgAN stratified according to the severity of the interstitial cellular infiltration found by light microscopy showed that the normal renal ICAM-1 expression on vascular endothelium was unchanged in the biopsies without interstitial cellular infiltration and with interstitial cellular infiltration of 1+. In IgAN with interstitial cellular infiltration ≥ 2+, moreover, ICAM-1 expression appeared on proximal tubule epithelium. The abnormal tubular expression of ICAM-1 was significantly associated with the degree of interstitial cellular infiltration and the number of interstitial leucocytes identified by MA. The biopsies with interstitial cellular infiltration confirmed the correlation between the CD45, CD14, and CD3 positive interstitial leucocytes and the quantitative tubular expression of ICAM-1. These results support the role of the tubular cells for interstitial leucocyte recruitment in IgAN, as has been shown in experimental GN [16,17].

In IgAN, both in synpharyngitic macrohaematuria episodes and during clinical quiescence, the level of circulating ICAM-1 is similar to that noted in healthy controls [18]. Thus, de novo expression of ICAM-1 on tubular epithelium seems a more specific local phenomenon than simply a reflection of the serum level. The association between ICAM-1 on proximal tubule epi-

Table 2. Median, range in brackets and significant Mann–Whitney U test of the number of interstitial leucocytes identified with monoclonal antibodies (MA) according to ICAM-1+ staining on proximal tubule epithelium (PTE). Spearman’s correlation coefficients between interstitial leucocytes and quantitative ICAM-1+ PTE in IgA nephropathy

<table>
<thead>
<tr>
<th>MA</th>
<th>ICAM-1+ PTE</th>
<th>ICAM-1− PTE</th>
<th>U test</th>
<th>ICAM-1+ PTEa (Quantitavely)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45</td>
<td>1088 (627–1127)</td>
<td>0 (0–932)</td>
<td>0.0003</td>
<td>r = 0.79b</td>
</tr>
<tr>
<td>CD18</td>
<td>545 (311–725)</td>
<td>0 (0–637)</td>
<td>0.0103</td>
<td>r = 0.69b</td>
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<tr>
<td>CD14</td>
<td>631 (0–1133)</td>
<td>0 (0–579)</td>
<td>0.0017</td>
<td>r = 0.67b</td>
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<tr>
<td>CD3</td>
<td>1016 (400–1809)</td>
<td>0 (0–900)</td>
<td>0.0001</td>
<td>r = 0.73b</td>
</tr>
<tr>
<td>CD4</td>
<td>664 (102–1150)</td>
<td>0 (0–625)</td>
<td>0.0001</td>
<td>r = 0.72b</td>
</tr>
<tr>
<td>CD8</td>
<td>387 (79–745)</td>
<td>0 (0–453)</td>
<td>0.0005</td>
<td>r = 0.68b</td>
</tr>
<tr>
<td>CD54</td>
<td>440 (48–1216)</td>
<td>0 (0–555)</td>
<td>0.0003</td>
<td>r = 0.59b</td>
</tr>
</tbody>
</table>

*aExpressed as mm² of ICAM-1+ proximal tubule epithelium per mm² of tubular cross-section.

bP < 0.001.

cP = 0.004.
Interleukin and interstitial cellular infiltrates in diverse GN [8,10,11], and particularly T lymphocytes and monocyte-macrophages as in our report, suggests that the tubular expression of ICAM-1 may be induced by interstitial cytokines in IgAN. In support of this mechanism, T-cells cloned from renal cellular infiltrates in experimental nephritis demonstrate specific autoreactivity against renal tissue and induce ICAM-1 on cultured tubular epithelial cells, the induction being blocked by a MA directed interferon-γ [19]. On the other hand, our study, which included 27 biopsies with proliferative GN, showed that the number of glomerular CD45+ (leucocytes) and CD3+ (T lymphocytes) was significantly higher in the biopsies with ICAM-1+ stain on proximal tubule epithelium than in the biopsies without, the correlation with the quantitative tubular expression of ICAM-1 being significant. These results suggest that glomerular cytokines [20] may be able to reach the tubulointerstitium via blood, urine, or diffusion through extravascular tissue, which would be a stimulus for the induction of ICAM-1 on tubular epithelium in IgAN.

A correlation between the number of CD8+ (cytotoxic/suppressor T cells) glomerular leucocytes and the quantitative ICAM-1 tubular expression was noted. In addition, the association between CD8+ cells infiltrating the interstitium and the presence of tubular ICAM-1+ staining are in accordance with the role of these cells in the interactions of ICAM-1 and its ligand LFA-1 (CD11a/CD18) showed in experimental GN [21].

There was a correlation between the number of tubular ICAM-1+ cells and the number of interstitial CD18+ leucocytes, which is in accordance with the ICAM-1/LFA-1 tubulointerstitial correlation reported in various types of human GN [22]. However, the better correlation between the number of interstitial ICAM-1+ cells and the number of interstitial CD18+ leucocytes infiltrating the interstitium noted in the biopsies with interstitial cellular infiltration suggest that the ICAM-1 mediated interactions are predominant within the interstitial infiltrate as has been observed in an experimental model [23]. Thus, tubular and interstitial ICAM-1+ could participate in adhesive interactions with leucocyte β2 integrin in the inflamed renal interstitium in IgAN.

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References

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