ICAM-1 expression in renal disease

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Abstract

Aim: To determine the role of intercellular adhesion molecule-1 (ICAM-1) in immune mediated damage in glomerulonephritis, and whether its expression correlates with disease activity.

Methods: Fifty three renal biopsy specimens from a range of non-immune renal disease and low grade and high grade glomerulonephritides were stained with ICAM-1. Positivity was assessed in the tubules. Tubular damage and accompanying interstitial inflammation were also noted. The ICAM-1 positivity in damaged and undamaged tubules was correlated with the three groups of renal disease.

Results: ICAM-1 positivity in undamaged tubules was observed in glomerulonephritis, and this showed a strong correlation with disease activity. In contrast, ICAM-1 positivity on damaged tubules correlated with evidence of chronic tubular damage, and was seen in a large proportion of cases, regardless of the underlying disease. There was no correlation between ICAM-1 positivity and a local lymphocytic infiltrate.

Conclusion: ICAM-1 probably has an important role in the pathogenesis of glomerulonephritis. Its expression may be secondary to cytokines released by cells participating in the glomerular damage. As these cytokines also influence tubule function, tubular ICAM-1 expression may be a marker of the extent of tubular disturbance in glomerulonephritis.

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Cellular adhesion molecules have an important role in the immune response by modulating cell-cell interactions after antigen presentation. Lymphocyte function associated antigen-1 (LFA-1) is one of many cellular adhesion molecules on lymphocytes. Studies on monoclonal antibody inhibition have shown that LFA-1 has a role in cytotoxic T lymphocyte mediated lysis,^{1,2} the proliferation of helper T cell populations,³ antibody responses to B lymphocytes⁴ and natural killer cell mediated cytotoxicity.¹

Intercellular adhesion molecule-1 (ICAM-1), a cell surface glycoprotein, has a role in the regulation of interactions among immune cells.^{5,6} This interaction with target cells is mediated through its ligand LFA-1.⁷⁻¹⁰ The key role of ICAM-1-LFA-1 interaction in the regulation of immune reactions is also shown

by the ability of cytokines such as tumour necrosis factor (TNF), interleukin-1 (IL-1), and γ -interferon (γ IF) to upregulate ICAM-1 expression.⁶

Previous studies of ICAM-1 expression in the kidney have demonstrated its role in T cell mediated rejection of allografts¹¹ and in murine lupus nephritis.¹²

Methods

Fifty three renal biopsy specimens were studied. The histological diagnoses were made on paraffin wax sections and by using immunofluorescence, together with relevant clinical information.

Frozen sections were cut and stained with mouse antihuman ICAM-1 (Serotec, Kidlington, Oxford) at a dilution of 1 in 200, using an indirect immunalkaline phosphatase technique. Negative controls were provided by exclusion of the primary antibody in the renal biopsy specimens and ICAM-1 staining of frozen sections of normal skin, in which keratinocytes do not stain with ICAM-1.¹³ The stained sections were examined and scored without knowing the clinical diagnosis. Positivity was assessed in the following structures: glomeruli, tubules, and blood vessels. The state of the tubules—that is, whether they were normal or exhibiting atrophy and peritubular fibrosis—was recorded. The presence of a lymphocytic infiltrate in the interstitium was also noted. The results of ICAM-1 positivity were then correlated with the clinicopathological diagnosis.

Tests for significance on proportions were done using the χ^2 test or Fisher's exact test where appropriate.

Results

The cases were subdivided on clinicopathological grounds into three categories: non-immune renal parenchymal disease (group 1, n = 14); low grade glomerulonephritis together with interstitial nephritis (group 2, n = 20); and high grade glomerulonephritis (group 3, n = 19). Details of these cases are listed in table 1.

Non-immune renal parenchymal disease included conditions such as acute tubular necrosis and ischaemic damage. The case of non-Hodgkin's lymphoma was not affected by tumour in the frozen tissue assessed.

Low grade glomerulonephritis and interstitial nephritis comprised the immune complex glomerulonephritides with a slow and variable course and two cases of interstitial

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Table 1 Histological diagnosis and tubule staining for ICAM-1

Group	n	ICAM-1 positivity on damaged tubules	ICAM-1 positivity on undamaged tubules
1 Non-immune renal parenchymal disease (n = 14):			
Hypertension	1	0	0
Thin membrane disease	1	0*	0
Acute tubular necrosis	3	1	0
Non-Hodgkin's lymphoma	1	1	1
Renal dysplasia	1	1	0
Reflux nephropathy	1	0	0
Diabetes mellitus	1	1	0
Amyloid	1	1	0
Ischaemia	3	2	0
Remnant nephropathy	1	1	0
2 Low grade glomerulonephritis and interstitial nephritis (n = 20):			
Minimal change disease	1	0*	1
Focal and segmental glomerulosclerosis	4	4	1
IgA nephropathy	4	2	0
Membranous glomerulonephritis	2	1*	1
Type 1 Mesangiocapillary glomerulonephritis	4	3	4
Mesangioproliferative GN (lupus II b)	1	0*	0
Mesangioproliferative GN	1	0*	0
Henoch-Schönlein purpura (no crescents)	1	0	0
Interstitial nephritis	2	0	0
3 High grade glomerulonephritis (n = 19):			
Henoch-Schönlein purpura (30-80% crescents)	3	1*	3
Diffuse proliferative crescentic GN	5	4*	3
Lupus nephritis (class IV) (4 crescentic)	7	4*	5
Type 1 Mesangiocapillary glomerulonephritis (crescentic)	1	1	†
Microscopic vasculitis with crescents	2	2	2
Haemolytic uraemic syndrome	1	1	0

* includes cases with no damaged tubules for assessment.
† undamaged tubules for assessment. GN = glomerulonephritis.

nephritis. The case of mesangioproliferative glomerulonephritis represented a recovery stage from a postinfectious proliferative glomerulonephritis a year earlier. Minimal change nephropathy was included in this group because, although the aetiology is uncertain, some evidence implicates a T cell disorder. High grade glomerulonephritis comprised cases with a rapidly aggressive clinical course,

and included all cases of crescentic nephritis, WHO class IV lupus nephritis, and a case of clinically and histologically extremely active haemolytic uraemic syndrome (HUS). ICAM-1 was consistently positive in the endothelium of small and medium sized vessels; this was used as the positive internal control (fig 1). The pattern was that of diffuse cytoplasmic staining. Cells within the glomeruli also stained, but it was difficult to differentiate the cell types on frozen sections (fig 1). Staining of the tubular epithelium was variable, depending on whether it was damaged, and the type of disease. Staining on undamaged tubules did indicate differences among the groups. The pattern of staining in all three groups was similar, showing a cytoplasmic positivity, mainly of the luminal portion of the tubular epithelial cells, and membrane staining of the luminal aspect. However, there were strong differences in the proportion of cases positive in the three groups (table 2). Only one case in group 1 showed positivity in the undamaged tubules; this was the case of non-Hodgkin's lymphoma (7%). In group 2 ICAM-1 positivity affected undamaged tubular epithelium in 35% of cases. This included a case each of minimal change disease, focal and segmental glomerulosclerosis, membranous glomerulonephritis, and four cases of type 1 MCGN (fig 2). The percentage of ICAM-1 positivity in low grade glomerulonephritis was significantly different from that in non-immune renal disorders ($p < 0.004$). In contrast, 72% of cases in group 3 showed ICAM-1 positivity in undamaged tubules (fig 3). This was significantly different from that in both the cases of non-immune renal diseases ($p < 0.002$) and low grade glomerulonephritis ($p < 0.02$). The percentage of cases exhibiting positivity

Figure 1 Endothelial cells in the peritubular vessels and medium size arteries are consistently positive with ICAM-1.

Figure 2 Undamaged proximal tubules in a case of crescentic diffuse proliferative glomerulonephritis shows cytoplasmic positivity with ICAM-1, mainly on the luminal side.

Figure 3 A case of type I mesangioproliferative glomerulonephritis exhibiting staining with ICAM-1 in the undamaged proximal tubules.

Table 2 ICAM-1 positivity in tubular epithelium in non-immune and immune-mediated renal disease

	Damaged tubules†	Undamaged tubules
1 Non immune renal parenchymal disease (n = 14)	8 (67%)	1 ^d (7%)
2 Low grade glomerulonephritis and interstitial nephritis (n = 20)	10 (71%)	7 ^a (35%)
3 High grade glomerulonephritis (n = 19)	13 (100%)	13 ^{a b c} (72%)

* one case did not have undamaged tubules for assessment.
† some cases did not have damaged tubules.
a 2 v 1 - p < 0.0004 (Fisher's exact test)
b 3 v 1 - p < 0.0002 (Fisher's exact test)
c 3 v 2 - p < 0.02 (χ^2 test)
d (3 + 2) v 1 - p < 0.0002 (Fisher's exact test).

in damaged tubules in non-immune renal parenchymal disease (group 1), low grade glomerulonephritis (group 2), and high grade glomerulonephritis (group 2) was 67%, 71%, and 100%, respectively (table 2). Hence damaged tubules, which were mainly atrophied with peritubular fibrosis, showed no significant difference in positivity among these three groups (fig 4). The positivity was that of a strong diffuse granular cytoplasmic pattern. The correlation of tubule ICAM-1 positivity with lymphocytic infiltration was assessed by first identifying the number of cases exhibiting both ICAM-1 tubule positivity and an associated increase in local lymphocytes and relating this to the number of cases showing either ICAM-1 positivity or a lymphocytic infiltrate. No lymphocytic infiltrate was seen in the vicinity of positively stained *undamaged* tubules in any of the groups.

In contrast, the percentage of cases exhibiting both positivity in *damaged* tubules and a lymphocytic infiltrate was 25%, 14%, and 23%, respectively, in groups 1, 2 and 3. The percentage of cases with positivity in damaged tubules (irrespective of lymphocytic infiltrate) was 67%, 71%, and 100%, in the three groups, respectively, while the percentages of cases with a lymphocytic infiltrate (irrespective of positivity in damaged tubules) were 36%, 20% and 21% in groups 1, 2, and 3, respectively. Thus the number of cases exhibiting both positivity in *damaged* tubules and a lymphocytic infiltrate was not greater than would be expected by chance. In particular, both cases of interstitial nephritis with a dense lymphocytic infiltrate were completely negative with ICAM-1. It therefore seems that ICAM-1 positivity in damaged tubules correlated with evidence of chronic tubular damage rather than an inflammatory infiltrate, and that ICAM-1 positivity in undamaged tubules was correlated with disease activity and not with the presence of local lymphocytes.

Discussion

In this study we found that 7% of biopsy specimens from cases of non-immune renal disease were positive with ICAM-1. Genetic make-up is a possible explanation for ICAM-1 expression in a few normal proximal tubules.¹¹ This differs from early studies of ICAM-1 expression in human tissues where proximal

Figure 4 A focus of atrophic tubules with peritubular fibrosis shows strong cytoplasmic positivity with ICAM-1. Note absence of a lymphocytic infiltrate in the interstitium.

tubules of kidneys were uniformly negative^{6,11} but is supported by the findings of Smith and Thomas,¹³ who demonstrated ICAM-1 expression in proximal tubules in the single piece of renal tissue included in their study.

Damaged tubules were frequently (67%–100%) positive and showed no correlation with disease activity. In contrast, ICAM-1 expression on undamaged tubules did relate to the underlying disease, with 35% of low grade glomerulonephritides and 72% of high grade glomerulonephritides being positive. The cases of glomerulonephritis exhibiting ICAM-1 positivity on undamaged tubules showed diffuse or focal segmental proliferation in the glomeruli, but no lymphocytic infiltrate was seen in the vicinity of these undamaged tubules. The correlation of ICAM-1 expression and disease severity is highlighted by the three cases of Henoch-Schönlein purpura with crescents which exhibited ICAM-1 positivity; a low grade Henoch-Schönlein purpura (histologically and clinically) was negative. In addition, five out of seven cases of class IV lupus nephritis exhibited ICAM-1 positivity in undamaged tubules whereas a case of class II lupus nephritis was negative.

It is uncertain if the cases of ICAM-1 expression in low grade glomerulonephritis represent an initial event in T cell activation, where the appearance of such lymphocytes is still not yet evident or in which cytokines may be released by circulating T lymphocytes. This would agree with Ooi *et al*'s findings of lymphocytotoxicity in patients with minimal change glomerulonephritis.¹⁴ The absence of a surrounding lymphocytic infiltrate in the vicinity

of these ICAM-1 positive tubules contradicts the findings of Weetman *et al* in autoimmune thyroiditis.¹⁵ The likely explanation for this is that ICAM-1 expression by thyrocytes renders them more susceptible to lymphocyte mediated cytotoxicity; but renal tubules are not the primary target of cell mediated lysis in glomerulonephritis. Both cases of interstitial nephritis examined were ICAM-1 negative.

Although there is no evidence of immunological damage to the tubules in cases of glomerulonephritis, the ICAM-1 expression none the less would be consistent with the release of cytokines in the microenvironment of the nephron as a result of primary glomerular immune complex disease. There is a body of evidence to suggest that the changes in tubular function, which accompany glomerulonephritis and other non-immune renal disorders, may be mediated by cytokines such as IL-1 and TNF, and also arachidonic acid metabolites.¹⁶ These factors alter the sodium-potassium-adenosinetriphosphatase pump and haemodynamics, and their presence would upregulate tubular ICAM-1 expression in glomerulonephritis. Because they are secreted by macrophages, it is not surprising that ICAM-1 expression correlates with proliferative activity in glomeruli and the presence of crescents. On this note, it is also interesting that although type 1 MCGN (without crescents) is a low grade disease, all the cases studied showed ICAM-1 positivity; this is presumably related to the mesangial proliferation and infiltration by macrophages.

The consistent presence of ICAM-1 in a significant proportion of damaged tubules which are atrophic with thickening of the tubular basement membrane and peritubular fibrosis is interesting. Inflammatory mediators released as a result of ischaemia may be the cause of this upregulation of ICAM.^{6,11} Alternatively, any damage promotes attempts at regeneration which may non-specifically upregulate a variety of surface molecules. Obviously, diagnostically, it is crucial to distinguish between this non-specific staining and the staining on undamaged tubules.

In conclusion, we have found that ICAM-1 is expressed in renal tubules in cases of glomerulonephritis, and that the degree of positivity correlates with disease activity. ICAM-1 expression on tubules does not correlate with the presence of an adjacent inflammatory infiltrate, raising the possibility that ICAM-1 expression may be secondary to cytokines released by cells participating in the glomerular damage. Because these cytokines also influence tubule function, tubule ICAM-1 expression may be a marker of the extent of tubular disturbance in glomerulonephritis.

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