GLomerular C3b RECEPTOR LOSS IN RENAL ALLOGRAFTS


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Summary

There is almost no data from the glomeruli of allografted kidneys with respect to changes in the CR-1 (C3b) receptor expressed on glomerular podocytes. We studied 22 renal graft biopsies from rejecting and stable allografts, using a panel of monoclonal antibodies. We found that the CR-1 expression was decreased in a focal and segmental fashion in some biopsies, particularly in rejecting kidneys. These changes correlated with the intensity of glomerular mononuclear cell infiltration, but in contrast no correlation was seen with peripheral capillary wall deposition of complement (C3). Thus, some active process is occurring in the glomeruli of rejecting grafts which affects the expression of the CR-1 receptor.

Introduction

In contrast to other mammals, human and other primate glomeruli express a receptor for the activated fragment of C3b [1], and these receptors are of the CR-1 type [2]. Similar receptors are present also on the surface of red blood cells, neutrophils, B-lymphocytes, macrophages and some T-lymphocytes [3]. The use of monoclonal antibodies, together with immunoelectron microscopic techniques, has demonstrated that their expression in the glomerulus is limited to the visceral epithelial cells (podocytes) [4].

The CR-1 receptors found outside the glomeruli are involved in the binding of immune complexes containing C3b, with subsequent endocytosis by fixed or migratory phagocytic cells [3]. The number of receptors expressed on the cell surface is variable, being increased in activated cells [3]. As with other surface receptors, they are also able to show the capping phenomenon [3]. The function of the CR-1 receptors in the glomerulus is, in contrast, unknown, but their expression is decreased in the glomeruli of patients with some types of glomerulonephritis [2,5]. This finding was at first thought to relate to the presence of deposits of C3 within the glomeruli [2,5], but this is now known to be incorrect: in lupus nephritis, the striking finding was that only in proliferative
glomerulonephritis was the expression of CR-1 decreased, even though there were extensive deposits of C₃ in the patients with membranous lupus nephropathy studied [5]. In most forms of proliferative glomerulonephritis, including lupus nephritis, monocytic infiltration is prominent [6].

In order to clarify a possible relation between the CR-1 receptor, glomerular complement deposition and mononuclear cell infiltration, we studied renal allograft biopsies obtained during the first few months after transplantation. It is now clear that mononuclear cells (particularly monocytes) invade the glomeruli of allografts, especially at times of rejection [7]. In contrast to glomerulonephritis, deposition of C₃ in the capillary walls is rare.

Patients and methods

Biopsies

Twenty-two needle biopsies were performed on 19 renal allografts, treated either with prednisone and azathioprine (10 biopsies) or prednisone and cyclosporine (12 biopsies). On clinical, radiological, radio-isotopic and histological criteria, 12 of the biopsies were considered to have been taken during episodes of rejection; the other biopsies are taken for episodes of renal dysfunction not considered to be the result of rejection, or routinely at one and four weeks in the case of the cyclosporine-treated grafts. Tissue was processed in all cases for conventional light microscopy, and in 12 biopsies was also studied using commercially-available peroxidase-conjugated antisera directed against IgG, IgM, IgA, C₃, C₄ and Clq.

Monoclonal antibodies

A panel of monoclonal antibodies, previously well-characterised, was applied to additional cryostat sections from all 22 biopsies and revealed using an indirect (anti-mouse) immunoperoxidase conjugate. The following antisera were used, recognising epitopes expressed on the cells indicated: all leucocytes (2D1); B-lymphocytes (TO 15); monocyte/macrophages (FMC 32); natural killer (NK) cells (Leu 7); Pan-T-cells (UCHT 1); T helper/inducer cells (Leu 3a); and T cytotoxic/suppressor cells (UCHT 4). For revealing C3b receptors, we used the antibody TO 5. Positive (tonsil) and negative controls were used with each monoclonal antibody. Only sections containing two or more glomeruli were reviewed. The total number of positive cells with each monoclonal antibody, and the number of glomeruli in each section was counted. Results were expressed as the number of positive cells per glomerulus. The intensity of the staining obtained with the anti-C3b monoclonal antibody TO 5, and with the polyclonal anti-C₃ and immunoglobulin antibodies was recorded.

Results

CR-1 staining

A total of 17 biopsies showed uniform peripheral staining of the CR-1 receptor, with a similar intensity in all, and identical to results obtained previously with
normal kidneys and from patients with interstitial nephritis (Figure 1). Although the CR-1 positivity appeared to be found only on the epithelial cells, we were unable, using light microscopy alone, to exclude endothelial or mesangial staining. An abnormal CR-1 pattern characterised by focal and segmental loss of the receptor was observed in five biopsies (Figure 2). Four of these were from rejecting grafts, and the fifth from a patient with cytomegalovirus (CMV) infection at the time of biopsy. The four biopsies represented 30 per cent (4/12) of those taken from rejecting kidneys.

Glomerular immune deposits

Of these five biopsies with abnormal CR-1 expression, glomerular immune deposits were sought in four: three rejecting kidneys, and the one with CMV. Only two of the rejecting kidneys showed any peripheral glomerular capillary fixation of anti-C₃ antibody, without immunoglobulins. The patient with CMV infection showed no C₃ fixation, but there was peripheral deposition of IgG, IgM and IgA. In the group of 17 patients with normal CR-1 expression in their biopsies, immune deposits were looked for in 11, but in only one was C₃ found
Figure 2. Immunoperoxidase staining of transplant glomerulus with a monoclonal antibody anti-C3b (TO 5). Cryostat section counterstained with haematoxylin. Magnification x 400 (reduced for publication). Areas with complete loss of the epithelial complement receptor are seen in the peripheral capillary walls, and immunoglobulins in two others, apparently linear in one.

**Intraglomerular mononuclear cells**

The numbers of intraglomerular cells in the biopsies with and without changes in expression of the CR-1 receptor are shown in Figure 3. Biopsies with CR-1 loss had a significantly higher number of intraglomerular leucocytes, with a predominance of macrophages. All types of cells were present in excess, however, except B cells and T helper/inducer cells (Wilcoxon rank-sum test).

**Effect of immunosuppressive regime**

The results appeared to be the same in patients given either azathioprine or cyclosporine as an adjunct to prednisone (Figure 1).

**Discussion**

Our results confirm that there is an increase in the number of infiltrating intraglomerular leucocytes, especially macrophages, and particularly in grafts judged
on other criteria to be rejecting. These grafts may show a decreased expression of the glomerular epithelial cell CR-1 receptor. The changes did not seem to correlate with the occasional presence of peripheral deposits of C₃, a finding
also noted in patients with glomerulonephritis [5]. Why and how the cell infiltration and the loss of CR-1 receptor are related is not known, or whether one is a primary and the other a secondary event. So far as we know, the glomerular podocyte does not have an immune function or phagocytic activity. Whether the CR-1 expression can be down-modulated by the infiltrating leucocytes, or whether the receptor shows capping and apparently decreased expression is also unknown, but both possibilities may operate. Whatever the link between the CR-1 changes and mononuclear cell infiltrate, these data are further evidence that in allografts, especially during rejection, an active process is taking place within the glomeruli, with macrophages playing an important role.

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References

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