Immunohistochemical Markers of Renal Tubular Injury and Cyclosporin Nephrotoxicity in Kidney Allograft Biopsies

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Dear Sir,

The differential diagnosis between renal allograft rejection and chronic cyclosporin (CS) nephrotoxicity presents serious difficulties for the histopathologist [1]. Vimentin expression in the cytoplasm of proximal tubule cells from drug-induced toxic nephropathies suggests that this intermediate filament could be used as a marker of both active cell regeneration and irreversible chronic tubular injury [2]. Chronic tubular injury has also been associated to changes in the expression of other antigens such as the epithelial membrane antigen (EMA), which is normally expressed by the distal tubule and collecting duct [3], and the CD15 antigen (Leu M1) on the proximal tubule brush border [4].

We have studied the expression of vimentin, EMA and CD15 in 48 renal allograft biopsies from patients subject to 2 different immunosuppressive protocols: prednisone plus low-dose CS (n = 31), and prednisone plus azathioprine (AZA; n = 17). Controls (n = 10) were obtained from otherwise normal kidneys removed after traumatic rupture. In the group treated with CS, a histopathological diagnosis of glomerulointerstitial allograft rejection was reached in 18 cases, while 5 of them showed chronic vasculointerstitial rejection, 4 acute interstitial rejection, 2 acute vascular rejection, 1 chronic transplant glomerulopathy and 1 recidivant focal and segmental hyalinosis. Among those patients treated with AZA, the most common histological diagnosis was chronic vasculointerstitial rejection (5 cases), followed by acute vascular rejection (4 cases), acute interstitial rejection (4 cases) and acute glomerulointerstitial rejection (4 cases). All biopsies were evaluated in a semiquantitative manner (0 = absent; 1 = mild; 2 = severe) for the presence of CS-associated changes including striped interstitial fibrosis, tubular calcifications, megamitochondria and hyaline droplets in the proximal tubules, peritubular capillary congestion, vascular myointimal fibrosis and hyaline arteriopathy. Ultrastructural confirmation of megamitochondria and hyaline droplets was performed in 50% of cases. The expression of vimentin, EMA and CD15 by the tubular cells was analyzed by avidin-biotin immunoperoxidase in paraffin-embedded tissue sections. The number of immunostained tubular profiles per 10 high-power (400 ×) microscopic fields was assessed.

Striped interstitial fibrosis and tubular megamitochondria and hyaline droplets were the changes significantly more common among the CS-treated patients than among those treated with AZA (59.25 vs. 38.46%, $p < 0.05$, for the presence of striped interstitial fibrosis, and 51.72 vs. 23.07%, $p < 0.05$, for the presence of megamitochondria and hyaline droplets; χ^2 test). On the other hand, myointimal fibrosis and hyaline arteriopathy were more common in the group treated with AZA than in those patients treated with CS (76.92 vs. 51.72%; $p < 0.05$), which correlates with higher incidence of vascular rejection in the AZA-treated group. Tubular atrophy was analyzed similarly by counting the number of profiles with thickening and/or splitting of the basement membrane and hyaline or granular casts. Both CS- and AZA-treated patients showed higher levels of expression of all 3 antigens (vimentin, EMA and CD15) than the controls (fig. 1a). CS-treated kidneys showed a significantly higher number of immunostained profiles than the AZA-treated ones, while atrophic tubules were more abundant in the AZA-treated group. Within the CS-

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$p < 0.01$

$p < 0.01$

$p < 0.05$

$p < 0.01$

0.01

Fig. 1. Immunohistochemical markers of tubular lesion. a Global series. b Cases with CS nephrotoxicity. TA = Tubular atrophy; VIM = vimentin; MNT = mean number of tubules per 10 high-power fields.

TA VIM EMA LeuM1 (CD15)

■ CS ■ AZA ■ Controls

17 b

treated group, 9 cases (23%) showed morphologic evidence of nephrotoxicity, with both striped interstitial fibrosis and tubular megamitochondria and hyaline droplets. The expression of vimentin and EMA were significantly higher in cases with CS toxicity (71.25 vs. 35.20%, $p < 0.01$, for vimentin immunostaining, and 185.88 vs. 132.72%, $p < 0.001$ for EMA immunostaining), while the expression of CD15 seemed to be lower in the CS nephrotoxicity group (fig. 1b).

Our findings that both AZA- and CS-treated allografts showed high levels of vimentin and EMA expression suggest that ischemia might be the basic mechanism responsible for this change in the tubular anti-

gen make-up. In fact, a considerable number of AZA-treated cases showed vascular and vasculointerstitial allograft rejection, and both acute and chronic vascular ischemia with progressive sclerosis have been shown in CS-treated allografts.

The immunohistochemical analysis of the expression of vimentin and EMA by the tubular cells of the renal allografts could be useful in the differential diagnosis between chronic vascular rejection and CS nephrotoxicity. In addition, a therapeutic switch to immunosuppression with AZA should be considered in those cases with highest levels of vimentin and EMA expression. Further studies, using experimental models of chronic CA nephro-

toxicity, are needed in order to confirm the diagnostic and therapeutic benefits of this type of analysis.

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