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Glomerular HLA DR DP DQ Expression in Renal Diseases

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Abstract: The expression of major histocompatibility antigens class II (MHC II) in renal diseases has been studied extensively in the tubulo-interstitial compartment, while there are few articles about their expression in the glomeruli. In this series, glomerular expression of HLA DR DP DQ is evaluated in a total of 62 cases of pyelonephritis, glomerulonephritis, tubulointerstitial nephritis and systemic diseases with renal manifestations in order to identify their relationship with disease status and renal scarring.

Formaline fixed paraffin embedded tissue sections from renal biopsy and nefrectomy specimens were stained by an antibody against HLA DR DP DQ by the streptavidin biotin method. Autopsy kidneys were used as control tissues. In order to understand the significance of glomerular HLA DR DP DQ expression in renal tissues, the results were correlated with glomerular sclerosis, interstitial fibrosis, tubular atrophy and interstitial inflammation by Spearman and Pearson correlation tests. Disease groups were compared with Kolmogorov-Smirnov tests in independent samples.

Increased expression was identified in cases with chronic pyelonephritis and membranoproliferative glomerulonephritis compared with autopsy kidneys. On the other hand, when all the cases were considered, no correlation was found between glomerular HLA expression and glomerular sclerosis ($p=0.458$, $r=-0.104$), tubular atrophy ($p=0.9$, $r=0.018$) interstitial fibrosis ($p=0.725$, $r=-0.049$) and inflammation ($p=0.987$, $r= 0.002$).

As there are few cases in each disease category, it is hard to reach definite conclusions about the different nature of HLA class II antigen expression in renal diseases. But their effect on renal scarring is not indicated by this study.

Key Words: HLA DR DP DQ, renal diseases, glomerular expression, immunohistochemistry, renal scarring.

Introduction

The major histocompatibility complex (MHC) is composed of three main HLA-class II loci, DR, DP and DQ. Their expression was first observed in B lymphocytes, followed by endothelial cells, T-cells and also epithelial cells in the tongue, tonsils, epiglottis, trachea, small intestine, urethra, epididymis and proximal tubular cells (1,2).

The tubular expression of HLA class II molecules has been extensively studied. It is known that HLA class II expression is increased in renal tubular epithelial cells both in renal allograft rejection and many forms of renal disease (2-7). On the other hand, there are few articles about the expression of HLA class II antigens in glomeruli in renal diseases (3,6,8). HLA class II antigens are important in antigen presentation. The expression leads to regional T-cell accumulation (9,10). T-lymphocytes play an important role in fibroblast proliferation through the secretion of growth factors like interleukin-4 (IL-4), tumour necrosing factor-α (TNF-α) and transforming growth factor-β (TGF-β) (11). TGF-β activates cell proliferation and migration of more T-cells (11,12). TGF-β is a fibrogenic cytokine and induces ECM synthesis, particularly of type I and III collagens (13). Like tubular cells, immune or non-immune injury to the glomerular cells may lead to MHC class II antigen expression, which is recognized by T-lymphocytes, and the IFN-γ secreted by the T-lymphocytes may increase MHC class II antigen expression (14,15).

The aim of the present study is to evaluate glomerular HLA DR DP DQ expression in autopsy kidneys and

pyelonephritis, glomerulonephritis, tubulointerstitial diseases and systemic diseases with emphasis on parameters of renal scarring such as glomerular, tubular and interstitial scarring, in order to highlight the role of this altered expression on renal scarring.

Materials and Methods

Five autopsy kidney specimens with normal renal morphology, 6 nephrectomy specimens with chronic pyelonephritis and 56 renal kidney needle biopsy specimens were evaluated for glomerular MHC class II expression and the results were correlated with interstitial inflammation, tubular atrophy, interstitial fibrosis and percentage of sclerotic glomeruli as well as primary diagnosis of the renal disease. Renal biopsy specimens have previously been evaluated by light microscopy and direct immunofluorescence (DIF). The distribution of the renal biopsies were as follows: IgA nephropathy (7 cases), membranoproliferative glomerulonephritis (MPGN) (11 cases), mesangioproliferative glomerulonephritis (MesGN) (3 cases), postinfectious glomerulonephritis (PIGN) (4 cases), crescentic glomerulonephritis (CrGN) (2 cases), minimal change disease (MCD) (2 cases), membranous glomerulonephritis (MGN) (14 cases), interstitial nephritis (IN) (3 cases), systemic lupus erythematosus (SLE) (4 cases), diabetic nephropathy (DN) (5 cases), chronic pyelonephritis (PN) (6 cases) and diffuse glomerulosclerosis (1 case).

Immunohistochemistry

The peroxidase labelled streptavidine biotine method (by Large volume DAKO LSAB II kit, peroxidase, DAKO Corp, Carpinteria, USA) was used along with the primary antibody against HLA DR DP DQ antigens (dil:1/50, DAKO Corp, Carpinteria, USA) for immunohistochemical staining. Tissue sections from formaline fixed paraffin embedded tissue blocks were taken on poly-l-lysine coated slides. Deparaffinisation was followed by rehydration in an alcohol series from 96% to 70%. Phosphate buffered saline solution (PBS) wash was performed for five minutes and it was repeated after every step. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Sections were boiled in PBS twice for 5 minutes in a microwave oven. Primary antibody was applied for 30 minutes. Biotinylated secondary antibody and streptavidin peroxidase solutions were applied for 10 minutes following PBS washes. Diaminobenzidine (Sigma Chemical Co, St. Louis, USA) was used as the chromogen. Sections were counterstained by Mayer's haematoxylin, and dehydrated in an increasing alcohol series.

Semi-quantitative evaluation

Immunohistochemical reaction intensity was evaluated in sections where at least 5 glomeruli could be observed. As endothelial cell staining with HLA class II antibodies is a constant finding observed in this study as well as in others, only glomerular mesangial and epithelial cells in the mesangial area were evaluated. Cytoplasmic and cell membrane staining was positive. Semiquantitative scores were given for glomerular HLA DR DP DQ expression as described before (3): No staining (0), a single positive cell (1), a few positive cells, expression by more than 10% and less than 50% of cells/glomeruli (2), many positive cells, more than 50% of cells/glomerulae (3) (Figure).

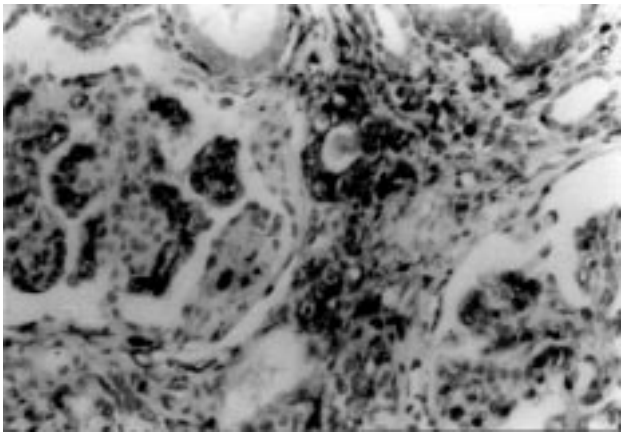

Figure 1. Glomerular HLA DR DP DQ expression in crescentic glomerulonephritis which was scored as 2 considering the group of mesangial cells with positive staining, while many cells at the other mesangial regions are negative. Note the few positive cells at the proliferative lesion at the Bowman's capsule, as well as tubular strong expression (DAB, IHCx200).

Morphometric evaluation of interstitial fibrosis

The point counting method was used for the determination of interstitial fibrosis in Masson's Trichrome stained sections as described by Howie et al. (16). Cortical areas adjacent to glomeruli were selected and observed at high power magnification. The images were obtained on a monitor (Sony) by a video camera (Sony) at 2100x magnification. A grid with 11 horizontal and vertical lines producing 121 intersecting points was put on the monitor. The points of intersection, which were

imposed on a green stained interstitium, were counted in 10 different areas by high power magnification. Points falling on other fields were also counted in the same areas, but glomeruli and vascular structures were excluded. The final result was found as follows:

$$\text{Interstitial fibrosis} = \frac{\text{number of points falling on interstitum}}{\text{number of points falling on cortical tissue (excluding glomeruli and vascular structures)}}$$

Quantification of sclerotic glomeruli

The proportion of sclerotic glomeruli to the number of all glomeruli in the tissue section was considered the glomerulosclerosis ratio.

Semiquantitative scoring of tubular atrophy and interstitial inflammation

Tubular atrophy was scored in periodic acid Schiff (PAS) stained sections while interstitial inflammation was scored in H&E stained sections by a modified scoring system adapted from Lajoe and Silva (17) as follows:

Scoring of tubular atrophy: 0: No atrophic changes; 1: atrophic changes in 20% of tubules; 2: atrophic changes in 20-40% of tubules; 3: atrophic changes in 40-70% of tubules; 4: atrophic changes in more than 70% of tubules.

Scoring of interstitial inflammation: 0: No inflammation; scores of 1, 2, 3 and 4 were given for cases with interstitial inflammation in 20%, 20-40%, 40-70% and 70-100% of the cortical interstitial area respectively.

Statistical Analysis

Statistical analysis was performed with Scientific Package for Social Sciences (SPSS) for Windows. Glomerular HLA DR DP DQ expression scores were correlated with morphometric interstitial fibrosis values, sclerotic glomeruli percentage, tubular atrophy and interstitial inflammation scores by Spearman correlation tests. All groups of cases with different histopathological diagnosis were compared with each other for HLA DR DP DQ expression by Kolmogorov-Smirnov tests in independent samples. Results with p*0.05 or p=0.05 were considered statistically significant.

Results

The glomerular HLA DR DP DQ antigen expression scores for the disease groups were as follows: IgA nephritis: 0-3 (mean 1.45); MPGN: 1-2 (mean 1.36); MesGN: 0-3 (mean 2.9); PIGN: 1-2 (mean 1.5); CrGN:1-2 (mean 1.5); MCD: 0-1 (mean 0.5); MGN: 0-2(mean 0.35); IN: 0-1 (mean: 0.33); SLE nephritis: 0-3 (mean:2); DN: 0-1 (mean: 0.8); chronic pyelonephritis: 0-2 (mean:1.5); and diffuse glomerulosclerosis: 1. The results for autopsy kidneys ranged from 0 to 1 (mean: 0.4) (Table).

The results of Kolmogorov-Smirnov Tests in independent samples comparing glomerular HLA scores in different disease groups were not statistically significant (p*0.05), but glomerular HLA scores were higher in cases of MPGN (p=0.003) and CP (p=0.044) than in autopsy kidneys.

Table. HLA DR DP DQ expression scores for renal diseases. Diffuse glomerulosclerosis with expression of HLA score: 1 is not shown in this table. (IgA: IgA nephritis, MPGN: memranoproliferative glomerulonephritis, Mes GN: mesangial proliferative glomerulonephritis, PIGN: postinfectious glomerulonephritis, CrGN: crescentic glomerulonephritis, MCD: minimal change disease, MGN: membranous glomerulonephritis, IN: interstitial nephritis, SLE: systemic lupus erythematosus associated nephritis, DN: diabetic nephropathy, CPn: chronic pyelonephritis, AU: autopsy kidneys.)

HLA Score	IgA	MP GN	Mes GN	PI GN	Cr GN	MCD	MGN	IN	SLE	DN	CPn	AU
0	3	-	1	-	-	-	10	2	1	1	1	4
1	-	7	-	2	1	1	3	1	2	3	4	1
2	2	4	-	2	1	1	1	-	-	1	1	-
3	2	-	2	-	-	-	-	-	1	-	-	-
total	7	11	3	4	2	2	14	3	4	5	6	5

When all the cases were considered, no correlation was found between glomerular HLA expression and glomerular sclerosis (p=0.458, r=-.0.104), tubular atrophy (p=0.9, r=0.018) interstitial fibrosis (p=0.725, r=-0.049) and inflammation (p=0.987, r= 0.002).

Discussion

There are controversial results about the distribution of glomerular MHC class II antigens in both normal and pathological renal tissues. The different antibodies used in different series rather complicate the issue. Daar et al. (1) noted MHC class II antigen expression constantly in glomerular mesangial and endothelial cells by NFK1 antibody in normal tissues. Müller et al. (3) used 4 primary antibodies against MHC class II antigens (TÜ22+DQ, TÜ34+DR, B7/21+DP, TÜ39+DR/DP/DY) and found TÜ39+DR/DP/DY expression in many glomerular cells and fewer cells with the other antibodies. They noted reduced HLA DR DP DY antigen expression in RPGN and reduced HLA DP and/or DR + cells in MesGN and MPGN although statistical analysis was not performed. In contrast with these results, in this series, MPGN cases were found to express increased HLA class II antigens, compared with autopsy kidneys. Although the antibody used in this study is not identical with the previous one, we think that reduced expression of HLA antigens in MPGN is a rather unexpected finding as mesangial proliferation and mesangial matrix expansion is a common feature for this disease group.

Wadgmayer et al. (6) stained normal renal biopsy specimens by anti HLA-DR antibody and stated that podocytes, mesangial cells and Bowman's capsule cells of normal glomeruli stained negative by HLA-DR antibody. They found increased glomerular HLA-DR antibody expression in cases with severe interstitial inflammation, as in transplant rejection, interstitial nephritis and lupus nephritis with extensive interstitial infiltrates. In this series 6 cases of CP were evaluated with rich interstitial infiltrates. Consistent with the findings of Wadgmayer et al. (6), in these cases increased HLA DR DP DQ antigen expression was observed, as compared to autopsy kidneys. On the other hand, by statistical analysis no correlation was found between interstitial inflammation and glomerular HLA class II antigen expression when all the cases were considered. But in this series, an antibody against HLA DR DP DQ antigens was used, which may be the reason for the controversial results.

In this study, statistical analysis comparing each disease group with the others and with autopsy kidneys was carried out. Except for the increased expression observed in MPGN and CP, none of the diseases were different from the others. These results may be affected by the low number of cases found in the disease groups and to the different stages of disease at the time of biopsy.

It is known that epithelial cells expressing MHC class II determinants can present antigens to cloned T cells. This is a very important step in the induction of both humoral and cellular immune responses (18). Li et al. (19) suggested that interaction of CD4 (+) T cell infiltration and intrinsic renal cells expressing MHC II is required for cell-mediated immune renal injury. This brings up the possibility of increased injury at sites with increased HLA class II antigen expression. If this is a major mechanism in renal scarring during renal diseases, one should expect an increased GS ratio for cases with increased HLA DR DP DQ expression. Also, interstitial fibrosis and tubular atrophy are frequently correlated with GS. Thus, on the whole, atrophic changes in three compartments should be expected. The attempt to find any correlation with glomerular HLA DR DP DQ expression scores and sclerotic glomeruli ratio, tubular atrophy and interstitial fibrosis failed in this series. These findings argue against the value of glomerular HLA DR DP DQ expression in progressing renal disease. Still, as the series includes many different diseases, it is hard to reach a definite conclusion when separate entities are considered.

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