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Increased glomerular Bax/Bcl2 ratio is positively correlated with glomerular sclerosis in lupus nephritis

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ABSTRACT

Introduction: The role of intrinsic pathway of apoptosis in pathogenesis of lupus nephritis (LN) is still not clear. We investigated the relation between the expression of two major proteins of the intrinsic pathway of apoptosis; bcl2 as an antiapoptotic protein and bax as a proapoptotic one; in renal tissue of LN.
Methods: The study included fifty paraffin embedded renal tissue obtained from renal biopsy specimens of LN patients (8 cases class II, 10 cases class III, 21 cases class IV and 11 cases class V) and five paraffin embedded apparently normal renal tissue obtained from nephrectomy specimens due to renal neoplasms as a control group. Immunohistochemical staining for bcl2 and bax antibodies was done. Ki67 immunohistochemical staining was done for class III and IV to assess the degree of proliferation. The number of intraglomerular bcl2, bax and ki67 positive cells per glomerular cross section was evaluated for each case. The results were analysed in different LN classes and correlated to different glomerular lesions. Results: The expression of bax and bcl2 proteins was higher in LN glomeruli compared to normal. The expression of bcl2 was significantly higher in class IV and was correlated to the degree of endocapillary hypercellularity. The bax to bcl2 ratio was significantly correlated to the percentage and degree of glomerular sclerosis.
Conclusion: The intrinsic pathway of apoptosis interfere in the pathogenesis of lupus glomerulonephritis. The balance between bax and bcl2 proteins might have a role in regulating the progression of glomeruli from proliferative to sclerotic state.

1. Introduction

Lupus nephritis is one of the serious manifestations of systemic lupus erythematosus. Many pathogenetic pathways participate in creating lupus nephritis pathophysiology [1–3]. Apoptosis is one of the pathways that is claimed to be involved in pathogenesis of lupus [4]. Sterner et al., [5] stated that lupus nephritis is associated with intraglomerular cell apoptosis which leads to release of nucleosomes (the fundamental unit of chromatin) that act both as inducer and target for autoantibodies. It is either dysregulated apoptosis or impaired clearance of apoptotic bodies that lead to exposure of nucleosomes and activation of antigen presenting cells and a T cell mediated response with formation of pathogenic immune complex inciting glomerulonephritis [4]. Disturbance in the process of apoptosis is now considered an important factor in development of systemic and organ-specific autoimmune diseases [6]. There are two major signaling pathways for induction of apoptosis; the extrinsic pathway controlled by death receptors of tumor necrosis factor receptors superfamily and the intrinsic pathway which is controlled by bcl2 family of proteins [7]. The intrinsic pathway of apoptosis plays important role as a checkpoint against development of autoimmune diseases. Overexpression of bcl2 in B cells of mice elicits an autoimmune response resembling systemic lupus erythematosus [8]. Also, mice deficient in the proapoptotic proteins bax and bak can lead to a fatal SLE-like autoimmune disease [9]. This emphasizes the role of these proteins in prevention of autoimmunity. Understanding the abnormalities in the expression of these proteins can be helpful in understanding the pathogenesis of lupus and guiding the therapy [10]. Wang et al., [11] found that inhibition

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of bcl2 protein in lupus nephritis-prone mice reduced the incidence of severe proteinuria, preserved the renal function and improved the overall survival.

2. Materials and methods

The study included fifty formalin fixed paraffin embedded blocks of tru cut needle biopsy of kidney tissue from patients with lupus nephritis who fulfill the criteria of The Systemic Lupus International Collaborating Clinics (SLICC) group [12]. Five formalin fixed paraffin blocks of morphologically normal renal tissue were obtained from normal renal tissue adjacent to renal neoplasms in nephrectomy specimens as a control group.

Microscopic examination of haematoxylin and eosin, periodic acid Schiff and masson trichrome stained sections were examined for detailed histopathological features. The specimens were classified according to International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 Classification of lupus nephritis [13] into eight cases of class II, ten cases of class III, twenty one cases of class IV and eleven cases of class V. Thus, we had thirty-one cases of proliferative LN and nineteen cases of non-proliferative LN.

Immunohistochemical staining for all cases by mouse monoclonal antibodies for Bcl2 (Genemed Biotechnologies, Inc) and Bax (Novus biological) using avidin-Biotin immunoperoxidase technique. Cases from class III and IV LN were stained by Ki67 using the same technique (Genemed Biotechnologies, Inc). Tissue sections of formalin fixed, paraffin embedded specimens were cut. Endogenous peroxidase was blocked by the use of Peroxidase Blocking Solution for 15 min. For antigen retrieval, the slides were immersed in citrate buffer (pH = 6) and put in the microwave for 9 min at 80 °C. After cooling, the slides were incubated overnight at room temperature with the diluted primary antibodies (1:25 for Bcl2 and 1:50 for Bax and Ki67). Then, Diaminobenzidine (DAB) chromogen solution were added and counter staining was done with Mayer's hematoxylin. With each run of staining, a positive control was included and a negative control, was done by omitting the primary antibody.

2.1. Immunohistochemical evaluation

In each case, the entire section was histologically examined by (×200 and ×400 magnification) to detect the site and distribution of staining for each antibody. The number of glomeruli in each section was assessed as well as the number of positive cells. The number of positive cells per glomerular cross section was calculated by dividing the number of positive cells per biopsy by the total number of non-sclerosed glomeruli in the same biopsy. The bcl2 to bax ratio was calculated by dividing the number of bcl2 positive cells per glomerular cross section by the number of bax positive cells per glomerular cross section. The statistical software package SPSS version 16 was used for all analysis.

3. Theory

We hypothesized that the expression of apoptotic proteins of the intrinsic pathway might have a role in pathogenesis of lupus nephritis and thus it might be related to the class of the disease and its activity. In our study we focused on the ratio between the immunohistochemical expression of bax and bcl2 proteins and the correlation between bax to bcl2 ratio and the active and chronic glomerular lesions in human LN. We hypothesized that the balance between these proteins might have an impact on the pathological changes that occur in LN.

4. Results

4.1. Bcl2 immunohistochemical staining

In morphologically normal renal tissue, bcl2 staining was noted within the epithelial cells of renal tubules and in the parietal epithelial cells of Bowman's space while it was absent within the glomerular tuft. In LN cases, bcl2 staining was noted in mesangial cells of glomerular tuft within the cellular crescents and within interstitial inflammatory cells, in addition to its presence within its normal location (Fig. 1). The mean number of intraglomerular bcl2 positive cells per glomerular cross section was significantly increased in class IV than other classes (Table 1 and Fig. 2). Also, intraglomerular bcl2 staining was significantly increased in biopsies which had endocapillary hypercellularity in more than 50% of the glomeruli (p value = .008). However, intraglomerular bcl2 staining was not affected by the presence of subendothelial deposits/hyaline thrombi/wireloop lesios or the degree of glomerular fibrinoid necrosis or leukocytic infiltration (Table 1). Intraglomerular bcl2 staining was also not correlated to percentage of sclerosed glomeruli (Table 2).

4.2. Bax immunohistochemical staining

Bax immunostaining was localized within the cytoplasm of renal tubular cells while it was absent in the glomeruli in the morphologically normal renal tissue. In LN cases, bax immunostaining was found within the glomeruli in mesangial, and visceral epithelial location and in crescents. Bax immunostaining was also found in interstitial inflammatory cells (Fig. 3). The difference in the mean number of intraglomerular bax positive cells per glomerular cross section between the classes was not significant and also it was insignificantly different in variable grades of all active glomerular lesions (Table 1). Bax immunostaining was also not correlated to percentage of sclerosed glomeruli (Table 2).

4.3. Bax to bcl2 ratio

The ratio between the intraglomerular bax stained cells per glomerular cross section and that of bcl2 (bax to bcl2 ratio) was not significantly different among LN classes. Bax to bcl2 ratio was also not significantly different with the difference in the degree of all active glomerular lesions. However, bax to bcl2 ratio had significant positive correlation with the percentage of sclerosed glomeruli (Table 2) and degree of glomerular sclerosis (Table 3 and Fig. 4).

4.4. Ki67 immunohistochemical staining

Immunohistochemical staining with ki67 antibody was performed on class III and IV LN (proliferative group) to assess the degree of proliferation. The mean number of intraglomerular stained nuclei per glomerular cross section was significantly higher in class IV than class III (Table 1) and It significantly increased with increasing degree of endocapillary hypercellularity; while it did not significantly differ with change in the degree of other active glomerular lesions (Table 1). However, the mean number of intraglomerular stained nuclei had significant inverse correlation with the percentage of sclerosed glomeruli per biopsy (Table 2). The mean number of intraglomerular ki67 stained nuclei was also significantly correlated to the mean number of bcl2 positive stained cells per glomerular cross section (r-value = 0.4, p-value = .02) but not to that of bax or the bax to bcl2 ratio.

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