

Enhanced major histocompatibility complex (MHC) Class II antigen expression in lupus nephritis

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Abstract

Thirty-eight cases of lupus nephritis, all satisfying the American Rheumatism Association criteria for diagnosis of systemic lupus erythematosus (SLE), with renal involvement and biopsy were immunohistochemically studied for the expression of HLA-DR (DAKO: HLA-DR/alpha, TAL.1B5), one of the three known families belonging to the class II major histocompatibility complex (MHC), using a standard **streptavidin-biotin-peroxidase** method. 20 nephrectomies performed for renal trauma and tumours constituted the normal controls. Of the lupus nephritis cases, 34 were females and 4 males. Ethnically, 20 were Chinese, 13 Malay, 4 Indian and 1 of indigenous origin. Their ages ranged from 16 to 59 years (mean of 31 years). Histologically, 23 expressed World Health Organisation (WHO) class IV (diffuse proliferative), 10 WHO class V (diffuse membranous), 4 WHO class II (pure mesangiopathy) and 1 WHO class III (segmental and focal proliferative) nephritis. Activity scores ranged between 5 to 19 (mean = 8.6) and chronicity scored between 2 to 7 (mean = 3.2) on a standard scoring system. Similar to other studies, HLA-DR was expressed in the glomerular capillaries and peritubular capillaries of all and mesangium, tubules (proximal, distal and collecting), veins and arterioles of some normal controls. Interestingly, HLA-DR expression was noted in the arteries of 25% of the normal controls, a finding hitherto not reported. The frequency of lupus nephritis cases expressing HLA-DR in the various anatomical components did not differ significantly from the normal controls except that HLA-DR expression in arteries and arterioles was seen at a significantly increased frequency ($p < 0.01$) in lupus nephritis. This increased expression did not correlate with the WHO class, activity or chronicity scores. It therefore appears that MHC class II shows increased expression in the arterial system of lupus nephritis kidneys. The significance of this is unclear but could be related to heightened (γ -interferon activation which may be a *de novo* phenomenon or result of T cell proliferation and activation in SLE.

Key words: Kidney, lupus nephritis, MHC class II, HLA-DR, immunohistochemistry, artery, arteriole.

INTRODUCTION

The kidney is involved in 90-95% of cases of systemic lupus erythematosus (SLE).¹ Hitherto, the pathogenesis of lupus nephritis remains unresolved although it is currently thought to be associated with chronic immune-complex deposition. In SLE, autoantibodies to self nucleoproteins, cytoplasmic and plasma membrane proteins as well as some plasma proteins have been identified.* However, the actual mechanism of autoimmune initiation is still unclear although several factors which include genetic susceptibility and oestrogen stimulation have been postulated.^{3,4} The major histocompatibility complex (MHC) class II antigens are known to be aberrantly expressed

in certain autoimmune diseases and have been implicated in the pathogenesis of these diseases.⁵⁻⁸ There has been limited work in this direction in lupus nephritis and results from studies on humans and animal models are scarce and controversial.^{9,10} Constituting 25% of the glomerulonephritides biopsied, lupus nephritis" is an important entity in Malaysians requiring more insight into its pathogenesis. An immunohistochemical study was conducted to map MHC class II expression in the anatomical components of lupus nephritis versus normal kidneys using a commercial monoclonal antibody to HLA-DR with the objectives of establishing the patterns of HLA-DR expression in normal kidneys and kidneys affected by lupus nephritis.

MATERIALS AND METHODS

Thirty-eight cases of lupus nephritis, histologically and immunohistochemically confirmed, were selected for study from the files of the Department of Pathology, University Hospital, Kuala Lumpur. All the cases satisfied the American Rheumatism Association criteria¹² for the diagnosis of SLE and had a minimum of 5 glomeruli in the biopsy. Histological categorisation was based on the World Health Organisation (WHO) classification system¹³ while activity and chronicity were assessed and scored using a standard scoring system.¹⁴ In addition, 17 nephrectomies performed for tumours and 3 for traumatic rupture were retrieved for use as normal controls. In this group, only cases in which there was normal kidney surrounding the localised pathology were selected and a paraffin block containing the most normal tissue was chosen for immunostaining.

One 4 µm section was cut from the formalin-fixed paraffin-embedded tissue block of all the lupus nephritis cases and controls on to aminopropyltriethoxysilane (TESPA) coated slides. The tissue sections were subjected to two rounds of microwave (Energy Beam Sciences, Inc., 600 watts, 100% power) antigen retrieval treatment at 100°C for 10 minutes each time prior to addition of the primary monoclonal anti-human HLA-DR antibody (DAKO: HLA-DR/alpha, TAL.1B5; dilution 1:100). The reaction was amplified by a commercial streptavidin-biotin/horseradish peroxidase kit (DAKO) and the final reaction product was visualised via 3,3'-diaminobenzidine tetrahydrochloride. Positive controls comprising of sections from a normal lymph node and negative controls from the same lymph node stained without the primary antibody were run with each batch. All cases were read by 2 renal pathologists separately. The results were charted for HLA-DR expression by anatomical component of both lupus nephritis cases and controls. Immunopositivity was only accepted in the presence of unequivocal staining and when both pathologists were in agreement to the findings. Results were statistically analysed by the chi-square test.

RESULTS

Of the 38 lupus nephritis cases, 34 were females and 4 male. Ethnically, 20 were Chinese, 13 Malay, 4 Indian and 1 of indigenous origin. Their ages ranged between 16 and 59 years

with a mean of 31 years. Histologically, 23 expressed Class IV (diffuse proliferative glomerulonephritis), 10 Class V (diffuse membranous glomerulonephritis), 4 Class II (pure mesangiopathy) and 1 Class III (segmental and focal proliferative glomerulonephritis) nephritis. All were active with the activity scores ranging from 5 to 19 (mean = 8.6) on a scale of 0 to 24. Chronicity scored between 2 to 7 (mean = 3.2) on a scale of 0 to 12. Table 1 charts the frequency and percentage of lupus nephritis cases and controls expressing HLA-DR immunopositivity by anatomical components. While the full range of anatomical components were identified in all the normal controls, distal tubules were identified in only 37, collecting ducts in 25, arteries in 37, arterioles in 17 and veins in 36 of the lupus nephritis biopsies. All lupus nephritis biopsies showed some mononuclear inflammatory interstitial infiltrate which uniformly expressed HLA-DR. Expression in the vascular system involved only the endothelium, sparing the rest of the vascular wall in both controls and lupus nephritis. The glomerular capillaries and the peritubular capillaries (Fig.1) of all lupus nephritis cases and controls expressed HLA-DR immunopositivity. In contrast, the podocytes (visceral epithelial cells) of both lupus nephritis and controls did not express HLA-DR. Expression in the mesangium, tubules (proximal, distal and collecting ducts) and veins was seen in a proportion of lupus nephritis and controls; the frequencies being not significantly different between the two. Conversely, HLA-DR was more frequently expressed in the arteries ($p < 0.01$) (Fig. 2) and arterioles ($p < 0.01$) of lupus nephritis kidneys compared with the controls. This increased frequency of expression did not show any correlation with the WHO class, activity or chronicity scores of the cases.

DISCUSSION

The major histocompatibility complex located on the short arm of human chromosome 6 encodes for at least three classes of antigens. Of these, class I and II are histocompatibility antigens best recognised for their involvement in transplant rejections. Class I antigens, especially HLA-A and -B, are ubiquitous and expressed on virtually all nucleated cells and platelets while expression of class II antigens (HLA-D) are generally restricted to cells of the immune system with certain exceptions. MHC class II antigens fall into three families, HLA-

TABLE 1: HLA-DR immunoreactivity in various anatomical components in lupus nephritis and normal kidneys

Anatomical component	Lupus nephritis No.positive/No.tested (% positive)	Normal No.positive/No.tested (% positive)
Glomerulus:		
Endothelium	38/38 (100.0)	20/20 (100.0)
Mesangium	30/38 (78.9)	15/20 (75.0)
Podocyte	0/38 (0)	0/20 (0)
Tubule:		
Proximal	32/38 (84.2)	12/20 (60.0)
Distal	20/37 (54.1)	7/20 (35.0)
Collecting	4/25 (16.0)	8/20 (40.0)
Vessels:		
Artery	32/37 (86.5)	5/20 (25.0)
Arteriole	13/17 (76.5)	2/20 (10.0)
Peritub cap*	38/38 (100.0)	20/20 (100.0)
Vein	35/36 (97.2)	20/20 (100.0)

* Peritubular capillary

DP, -DQ and -DR with HLA-DR currently having the largest number of recognised serological specificities. Although HLA-DR antigens are polymorphic, this being conferred by the beta chains, they share a monomorphic alpha-chain to which the monoclonal antibody DAKO:HLA-DR/alpha, TAL.1B5 reacts." Immunohistochemical detection using TAL.1B5 therefore provides a reasonable representation of MHC class II expression.

As noted in earlier studies,¹⁶⁻¹⁸ this study confirms that HLA-DR is normally expressed in glomerular and peritubular capillaries, mesangium, tubules, veins and arterioles. Podocytes do not express HLA-DR. Interestingly and unlike the findings of other workers,¹⁶⁻¹⁸ HLA-DR expression was observed in the arteries of 5 (25%) of the 20 normal controls. In the lupus nephritis group, HLA-DR expression resembled the controls in being observed in all the above stated anatomical components except the podocyte. No significant difference was noted in the frequency of HLA-DR expression in the glomerular capillaries, peritubular capillaries, mesangium, tubules and veins of controls and lupus nephritis cases. However, HLA-DR expression was observed in the arteries of 86.5% of lupus nephritis cases compared to 25% ($p < 0.01$) of controls. Expression was observed in the arterioles in 76.5% of lupus nephritis cases and 10.0% (p

< 0.01) in normal controls. This points to an enhanced MHC class II expression in the arterial system in lupus nephritis. Although the reason for this is not immediately evident, it is notable that this finding has not been previously reported. Considerations for this phenomenon should include whether it is of primary aetiological importance to or a result of the autoimmune disease. In favour of the latter would be that lupus nephritis is part of a systemic disease in which a single organ such as the kidney would seem an unlikely source and cause of the disease. Secondly, MHC class II antigens are induced by γ -interferon, a cytokine produced by activated T cells; increased circulating numbers of which occur in active SLE.¹⁹ This would infer that increased arterial MHC class II expression in lupus nephritis is more likely to be a result rather than cause. The implication that MHC class II is the result of γ -interferon induction is also suggested in a recent study on the MRL-lpr animal model of lupus nephritis which also proposes a genetically determined heightened γ -interferon status as the causative mechanism rather than the resultant effect of lupus nephritis.²⁰ The finding of a similar pattern of MHC class II expression in normal controls and lupus nephritis, albeit at a lower frequency in the arterial system in the control group, would also suggest that the expression in lupus nephritis is enhanced but not aberrant,

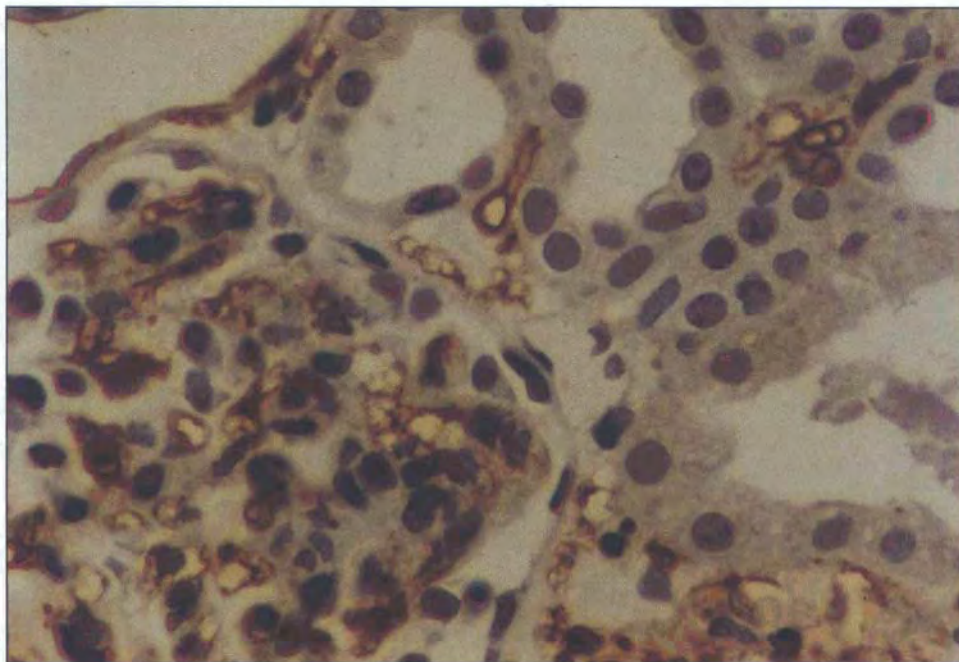


FIG. 1: Expression of HLA-DR by the endothelial cells of peritubular capillaries and glomerular capillaries of a case of proliferative lupus nephritis. (Immunoperoxidase stain $\times 800$)

implying that it is unlikely to be causally related in the pathogenesis of lupus nephritis. Nevertheless, the role of this enhancement is still unclarified. It can however be assumed

that the enhanced and more widespread MHC class II expression in lupus nephritis would augment the immune response via the increased ability of CD4+ (helper) T cells to recognise

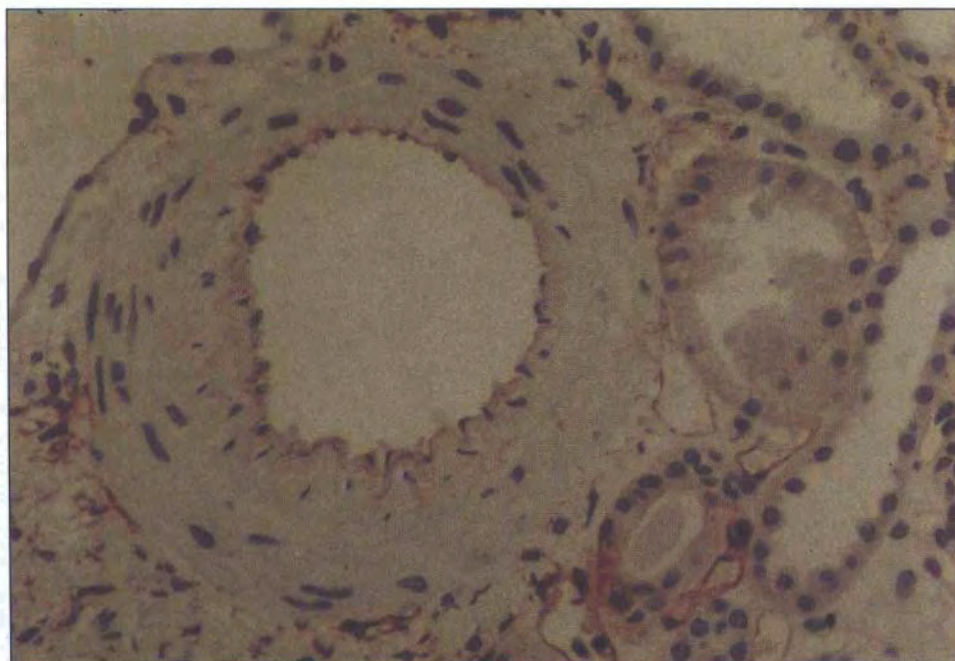


FIG. 2: HLA-DR expressed by the arterial endothelial cells of a proliferative lupus nephritis case. (Immunoperoxidase stain $\times 400$)

antigens, since CD4+ T cells only recognise antigens in the presence of MHC class II antigens. Although the lack of correlation between the increased arterial HLA-DR with the WHO class, activity and chronicity scores leads to some hesitancy to this postulation, accepting that one or more of these factors should provide an indication of immunological activity in the kidney, systemic activity which was not assessed in this study could have contributed significantly to γ -interferon production and subsequent MHC class II expression. As an adjunct, it would also be interesting to study the extent of arterial MHC class II expression in SLE and whether it is associated with clinical activity.

The finding of MHC class II antigen expression in arteries of normal controls has also not been documented previously.¹⁶⁻¹⁸ Whether this suggests a genetic difference, an increased prevalence of subclinical lupus nephritis or a higher baseline γ -interferon level resulting from some other reason in the Malaysian population requires further study. Notwithstanding, it has to be taken into account that pre-transplant grafts were used as "normals" by most workers,^{16,17} while normal kidney around tumours and traumatic rupture were utilised in this study. It may be pertinent to note that of the 5 normal control cases in which HLA-DR expression was noted in the arteries of the normal kidney tissue, 4 were surrounding malignancies (2 renal cell carcinomas, 1 Wilms' tumour and 1 metastatic endometrial carcinoma). Similarly both cases of arteriolar HLA-DR expression in this group came from nephrectomies performed for renal cell carcinoma. Although HLA-DR expression has not been documented in the arterial system of normal kidneys, HLA-DR expression has been noted in the neoplastic cells of certain malignancies including renal cell carcinomas, some childhood tumours and adenocarcinomas.²¹⁻²³ While it may be argued that the arterial and arteriolar HLA-DR expression in the normal kidney tissue of the controls may be associated in some way with the adjacent malignancy, the neoplastic tissue per se of these cases did not exhibit any immunohistochemically detectable HLA-DR positivity, hence lessening the likelihood of such a possibility.

In summary, it appears that most parts of the kidney can express MHC class II antigens. Interestingly, even the highly resistant human podocyte has been noted to express MHC class II antigens under γ -interferon induction.²⁴ This widespread antigen-presenting system to CD4+

T cells may explain for why the kidney is a haven for immunological events and perhaps account for the large number of immunologically driven glomerulonephritis.

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REFERENCES

- Hill G. Systemic lupus erythematosus and mixed connective tissue disease. In: Heptinstall RH, ed. Pathology of the kidney. 4th ed. Boston, Toronto, London: Little, Brown and company, 1992; 886.
- Dixon FJ. The pathogenesis of murine systemic lupus erythematosus. Am J Pathol 1979; 97:10-6.
- Gibofsky A, Winchester RJ, Patarrogo M. Disease associations of Ia-like alloantigens: Contrasting patterns in rheumatoid arthritis and systemic lupus erythematosus. J Exp Med 1978; 148:1728-32.
- Melez KA, Reeves JP, Steinberg AD. Regulation of the expression of autoimmunity in NZB X N2W F1 mice by sex hormones. J Immunopharmacol 1979; 1:27-34.
- Matsumoto Y, Hara N, Tanaka R, Fujiwara M. Immunohistochemical analysis of the rat central nervous system during experimental allergic encephalomyelitis, with special reference to Ia-positive cells with dendritic morphology. J Immunol 1986; 136:3668-76.
- Londei M, Lamb JR, Bottazzo GF, Feldmann M. Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. Nature 1984; 312:639-41.
- Teyton L, Lotteau V, Turmel P, Arenzana-Seisdedos F, et al. HLA DR, DQ, DP antigen expression in rheumatoid synovial cells: a biochemical and quantitative study. J Immunol 1987; 138:1730-38.
- Bottazzo GF, Dean BM, McNally JM, MacKay EH, Swift PGF, Gamble DR. In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulinitis. N Engl J Med 1985; 313:353-60.
- Wuthrich RP, Yui MA, Mazoujian G, Nabavi N, Glimcher LH, Kelley VE. Enhanced MHC Class II expression in renal proximal tubules precedes loss of renal function in MRL/lpr mice with lupus nephritis. Am J Pathol 1989; 134: 45-51.
- Yokoyama H, Takabatake T, Takaeda M, et al. Up-regulated MHC-class II expression and γ -IFN and soluble IL-2R in lupus nephritis. Kidney Int 1992; 42: 755-63.
- Looi LM. The pattern of renal disease in Malaysia. Malays J Pathol 1994; 16:19-21.
- Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982; 25:1271-7.

13. **McCluskey** RT. Lupus nephritis. In: Summers SC, ed. *Kidney pathology: Decennial*. New York: **Appleton & Lange**, 1975:456-9.
14. Austin HA, Muenz LR, Joyce KM, Antonovych **TT**, **Barlow** JE. Diffuse proliferative lupus nephritis: Identification of specific pathological features affecting renal outcome. *Kidney Int* 1984;25:689-95.
15. Adams TE, Bodmer JG, Bodmer WF. Production and characterization of monoclonal antibodies recognizing the alpha-chain **subunits** of human Ia alloantigens. *Immunology* 1983; 50:613-24.
16. Fuggle SV, Errasti P, Daar AS, Fabre JW, Ting A, **Morris** PJ. Localization of Major Histocompatibility Complex (HLA-ABC and DR) antigens in 46 kidneys. *Transplantation* 1983; 35:385-90.
17. Evans PR, Trickett LP, Smith JL, **MacIver** AG, Tate D, Slapak M. Varying expression of major **histocompatibility** complex antigens on human renal endothelium and epithelium. *Br J Exp Path* 1985; 66:79-87.
18. **Hinglais** N, Kazatchkine MD, Charron DJ, *et al*. Immunohistochemical study of **Ia** antigen in the normal and diseased kidney. *Kidney Int* 1984; 25:544-50.
19. **Erkeller** YF, **Hulstaart** F, Hannet I, **Isenberg** D, Lydyard P. Lymphocyte subsets in a large cohort of patients with systemic lupus erythematosus. *Lupus* 1993; 2: 227-31.
20. Balomenos D, **Rumold** R, Theofilopoulos AN. Interferon-gamma is required for lupus-like disease and lymphoaccumulation in **MRL-lpr** mice. *J Clin Invest* 1998; 101: 364-71.
21. Markovic-Lipkovski J, Brasanac D, Todorovic V, **Muller** CA, **Muller** GA. Immunomorphological characteristics of renal cell carcinoma. *Histol Histopathol* 1995; 10: 651-9.
22. **Vanky** F, **Klein** E, **Willems** J. DR antigens expressed on tumour cells do not contribute to the blastogenetic response of autologous T cells. *Cancer Immunol Immunother* 1985; 219-25.
23. Pilkington GR, **Pallesen** G. Phenotypic characterisation of non-haemopoietic small cell tumours of childhood with monoclonal antibodies to leucocytes, **epithelial** cells and cytoskeletal proteins. *Histopathol* 1989; 14:347-57.
24. Baudeau C, Delarue F, He CJ, *et al*. Induction of MHC class II molecules HLA-DR, -DP and -DQ and **ICAM 1** in human podocytes by **gamma**-interferon. *Exp Nephrol* 1994; 2: 306-12.