Journal of Korean Medical Science
Vol. 1, No. 1, P 43-48. Sep. 1986

Immunological Abnormalities in Patient with IgA Nephropathy

Chun Gyoo Ihm, M.D., Jeong Taek Woo, M.D., Young Woon Chang, M.D.,
O Sun Kwon, M.D. and Myung Jae Kim, M.D.

[1]*Department of Internal Medicine, College of Medicine, Kyung Hee University Seoul, Korea*

T cell immunity and phagocytic activity were studied in the blood of patients with IgA nephropathy in order to clarify their roles in the pathogenesis of IgA nephropathy. The percentages of total T lymphocytes, helper T cell and suppressor T cells were significantly reduced in patients. A significantly elevated helper T cell/suppressor T cell ratio in patients showed a predominant reduction in suppressor T cells. There was a significant relationship between histologic findings and helper T cell/suppressor T cell ratio in patients. Natural Killer (NK) cell activity was significantly reduced but the lymphocyte response after phytohemagglutinin (PHA) stimulation was not in patients. ConA-induced suppressor cell activity was not depressed despite of a decrease in suppressor T cells in patients. Phagocytic activity of polymorphonuclear leucocytes (PMNs) ingesting yeasts was significantly reduced in patients. Also an inverse correlation was found between serum IgA levels and phagocytic activity of PMN. It is concluded that suppressor T cell defects, depressed phagocytic activity and impaired NK cell activity may play a role in the pathogenesis of IgA nephropathy.

Key Words: *IgA nephropathy, helper T cell, suppressor T cell, Natural Killer (NK) cell activity.*

INTRODUCTION

IgA nephropathy is a widely recognized entity characterized by the presence of prominent IgA deposits in the mesangium in the absence of systemic diseases (Berger, 1979). It is recognized as the most common form the glomerulonephritis in France, Australia, Japan and Korea (Clarkson et al., 1984; Lee et al., 1985).

The pathogenesis of IgA nephropathy is unknown, and several hypotheses have been proposed to explain the occurrence of nephritogenic IgA class immune complexes (Clarkson et al., 1984). ConA-inducible suppression of IgA synthesis has been reported and there have been a few studies on phagocytic cells in the clearance of IgA immune complex. Information about involvement of the NK cell is lacking. Therefore we investigated T cell immunity and phagocytic activity in order to clarify their roles in the pathogenesis of IgA nephropathy.

Address for Correspondence: *Myung Jae Kim M.D., Department of Internal Medicine, College of Medicine, Kyung-Hee University, Hoe Ki-Dong, Dongdaemun-Gu,, Seoul, 131, Korea.*

SUBJECTS AND METHODS

17 patients with IgA nephropathy were studied. Blood samples from 18 healthy donors were obtained as normal controls. Peripheral blood mononuclear cells (PBMs) were prepared from heparinized blood by the standard Ficoll-Hypaque gradient method.

The T lymphocytes, helper T (T_M or Tu) cells and suppressor T (T_G or Tr) cells were quantitated by the double rosette method (Lim et al., 1985), and the B lymphocytes by erythrocyte antibody complement rosette method (Basten et al., 1972).

T_M or T_G cells were demonstrated with ox RBC or guinea pig RBC coated with IgM or IgG antibody to the erythrocyte membrane. PHA stimulation was determined with different concentrations of 50 ug/ml and 100 ug/ml.

NK cell activity of PBMs was assayed with ^{51}Cr-

labeled K 562 cells as targets similar to those described preciously (Raska et al., 1982). NK cell activity was calculated as;% NK cell activity

$$= \frac{\text{CPM (test)} - \text{CPM (spontaneous)}}{\text{CPM (total)} - \text{CPM (spontaneous)}} \times 100$$

Phagocytic activity of PMNs was assayed with yeasts or erythrocyte antibody complement (EAC). 200 PMNs were counted and the ingestion rate was calculated from the following formulae;

$$\% \text{ ingestion rate} = \frac{\text{PMN containing yeasts or EAC}}{200 \text{ PMN}} \times 100$$

ConA-induced suppressor cell assay is similar to those described previously (Lortan et al., 1982; Sakai, et al., 1979). PBMs were cultured for 48hr at 37°C in the presence or absence of ConA 30 ug/ml. Cultured PBMs were centrifuged and the supernatant was obtained. Normal PBMs from a single donor were used as responder cells to stimulation by PHA 20 ug/ml. The effect of patient supernatant, both ConA stimulated and nonstimulated, on the transformation of normal responder lymphocytes was measured by incorporation of ^{14}C-thymidine after a 72-hr incubation. The percentage suppression of mitogen response was calculated as follows;

$$\% \text{ ConA suppression} = 1 - \frac{\text{CPM ConA-stimulated cells}}{\text{CPM non-stimulated cells}} \times 100$$

RESULTS

Lymphocyte Subpopulations; The percentages of T lymphocytes, B lymphocytes, T_G and T_M cells in total lymphocyte population are shown in Table 1. The average relative proportions of total T lymphocytes, T_M and T_G cells were significantly reduced in patients with IgA nephropathy ($P < 0.005$). These patients showed a significantly elevated T_M/T_G cell ratio as compared with control subjects ($P < 0.005$); r11.5±10.6 and 5.4±1.1 (S.D.), respectively. The relative proportions of B lymphocytes were not different between patients and control subjects.

NK Cell Activity; The spontaneous cytotoxicity of PBM was significantly reduced in patients as compared with control subjects ($P < 0.05$); 43.5±20.1% and 57.8±14.5% (S.D.), respectively (Table 1).

Mitogenic Lymphocyte Response in Vitro; In the polyclonal stimulation with PHA, the mean responses of lymphocytes were not different between patients and control subjects (Table 2).

Phagocytic Activity of PMN; Phagocytic activity of PMNs ingesting yeasts in patients was significantly reduced as compared with control subjects ($P < 0.05$); 58.6±14.2% and 70.7±6.7% (S.D.), respectively (Table 3, Fig. 1). mean phagocytic activity of PMNs ingesting EAC (similar to immune complex) was not different between patients and control subjects.

ConA-induced Suppressor Cell Assay; Suppressor cell activity in patients was not different from that in control subjects (Table 4).

Relationship between the Immune Parameters and the Clinicopathologic Findings; The extent of phagocytic activity or T_M/T_G cell ratio was compared with serum IgA levels, the degree of hematuria and the histologic findings including the presence or absence of immune deposits along the capillary walls (Table 5,6,7).

Table 1. Total T cells and B cells, suppressor T cells, helper T cells and NK cell activity in patients with IgA nephropathy

	Patient with IgA nephropathy	Control (n = 18)	Significance
T cell %	56.7 ± 6.7 (n = 14)	67.0 ± 6.0	$p < 0.005$
B cell %	10.9 ± 4.9 (n = 11)	15.0 ± 3.0	NS
T_G %	4.4 ± 2.2 (n = 17)	8.3 ± 1.3	$p < 0.005$
T_M %	34.8 ± 10.3 (n = 16)	43.9 ± 3.7	$p < 0.005$
T_M/T_G ratio	11.5 ± 10.6 (n = 16)	5.4 ± 1.1	$p < 0.005$
NK cell activity %	43.5 ± 20.1 (n = 12)	57.8 ± 14.5	$p < 0.05$

Mean ± S.D. NS: Not significant

Table 2. PHA stimulation test in patients with IgA nephropathy. (Mean ± S.D.)

PHA conc. (μg/ml)	Patients with IgA Nephropathy (× 10^3 CPM)	Control (× 10^3 CPM)
50	137 ± 36	105 ± 59
100	199 ± 21	193 ± 46

Table 3. Phagocytic activity of PMN in patients with IgA nephropathy.

	Patients with IgA nephropathy	Control	Significance
Yeast	58.6 ± 14.2% (n = 8)	70.7 ± 6.7% (n=7)	$P < 0.05$
EAC	85.0 ± 6.8% (n = 7)	84.1 ± 7.5% (n = 8)	NS

NS: Not significant
Mean ± S.D.

Table 4. Con A-induced suppressor cell activity in patients with IgA nephropathy.

	Patients with IgA nephropathy (n = 6)	Control) (n = 7)
Suppressor cell activity (%)	27.0 ± 17.7	29.9 ± 10.9

Mean ± S.D.

Table 5. Relationship between the clinicopathologic findings and T_M/T_G ratio.

	No. of cases	T_M/T_G ratio	Significance
Gross hematuria	8	11.7 ± 9.1	NS
Microscopic hematuria	8	11.1 ± 10.34	
Minimal change	2	3.0 ± 0.4	$P < 0.01$
MsPGN and FSGS	12	8.2 ± 5.2	
Capillary wall (+)	5	10.1 ± 11.7	NS
deposit (−)	10	8.9 ± 7.1	

NS: Not significant
Mean ± S.D.

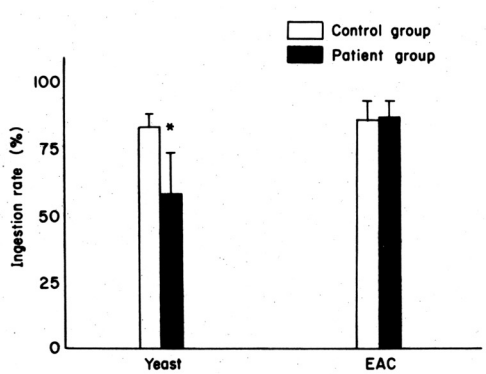

Fig. 1. Phagocytic activity of PMN in patients with IgA nephropathy.
*P < 0.05 vs. normal control

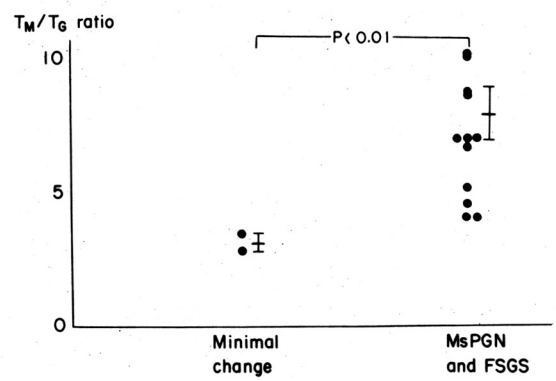

Fig. 2. Relationship between T_M/T_G ratio and histologic findings in IgA nephropathy (Mean ± S.E.).

T_M/T_G cell ratio was significantly elevated in patients with more severe histologic findings such as mesangial proliferation and focal or segmental sclerosis as compared with minimal change (P 0.01) (Table 5, Fig. 2). Also an inverse correlation was found between serum IgA levels and phagocytic activity of PMN ingesting EAC (r = 0.88, P 0.05) (Table 7).

Table 6. Relationship between the clinicopathologic findings and phagocytic activity.

	Phagocytic activity	
	Yeast	EAC
Gross hematuria	66% (n = 2)	88% (n = 3)
Microscopic hematuria	58% (n = 6)	82% (n = 4)
Capillary wall (+)	79% (n = 1)	86% (n = 2)
deposit (−)	56% (n = 5)	82% (n = 4)

Table 7. Correlation coefficients between immune parameters and serum IgA levels.

	Serum IgA levels	Significance
T_M/T_G ratio	0.2 (n = 15)	NS
Phagocytic activity		
Yeast	0.08 (n = 5)	NS
EAC	−0.88 (n = 5)	$P < 0.05$

DISCUSSION

The diagnostic features of primary IgA nephropathy are mesangial deposits of IgA, usually accompanied by C_3, mesangial expansion and proliferation, and electron dense mesangial deposits on the exclusion of alcoholic cirrhosis, systemic lupus erythematosus and Henoch-Schoenlein purpura. Long-term follow-up studies have not confirmed initial impressions that IgA nephropathy is benign (Clarkson et al., 1984; Lee et al., 1985). Several groups have now recorded that 10-30% of patients progress to renal failure and the incidence of hypertension is high.

The pathogenesis of IgA nephropathy is as yet unknown, but several hypotheses have been proposed to explain the occurrence of nephritogenic IgA class immune complexes (Clarkson et al., 1984). The first presupposes a defect in antigen exclusion at the mucosal level allowing increased antigen entry.

The second involves decreased immune clearance. For example, IgA deposits have been recognized in patients with alcoholic cirrhosis (Callard et al., 1975) and may be due to decreased activity of hepatic Kupffer cells. Mesangial cells appear to have a phagocytic capacity like RES. On the other hand, mechanisms for removal of IgA class immune complexes from the circulation are unclear. It has been reported that receptors for polymeric IgA are present on the PMN membrane (Lawrence et al., 1975). Others suggested involvement of tissue macrophages and monocytes in the clearance of IgA immune complexes (Roccatello et al., 1984). But another study reported normal splenic Fc-receptor function and hepatic C3b-receptor function by measurement of their blood clearance in mesangial proliferative glomerulonephritis including IgA nephropathy. (Solomon et al., 1984) In our study, PMN phagocytic function was demonstrated with yeast or erythrocyte-antibody-complement similar to recently developed immunobeads (Sato et al., 1983) The phagocytosis of yeast by PMN was reduced, but phagocytosis of EAC was not in patients. There were no correlations between this depressed activity and degree of hematuria and the presence or absence of immune deposits along the capillary wall. But this depressed activity was inversely correlated with serum IgA levels. Earlier study (Sato et al., 1983) using immunobead similar to EAC showed the depressed phagocytic activity in patients and reported that a significant correlation existed between the ingestive function of PMN and glomerular histological abnormalities. Therefore, although earlier study reported that binding of IgA-containing immune complexes leads to PMN dysfunction (Wilton, 1978), this depressed phagocytic activity of PMN may cause mesangial IgA deposition.

In further studies evaluating the phagocytic function, the method using purified IgA-containing immune complex may be necessary.

Furthermore, as we proposed (Woo et al., 1985), because levamisole among many immune modulators increases phagocytosis, the pathologic follow-up study will be required to elucidate the effect of this drug on IgA nephropathy showing depressed phagocytic activity.

The third mechanism is perhaps of most relevance to IgA nephropathy and involves a defect in the regulation of IgA production, such as T cell dysfunction. Our study clearly indicated that these patients have a significantly elevated helper T cell/suppressor T cell ratio due to a predominant reduction in suppressor T cells in agreement with other reports (Bannister et al., 1983; Egido et al., 1983; Chatenoud & Bach. 1981) But ConA-induced suppressor cell activity in patients were not different from those in normal controls.

Recent studies of in vitro IgA production or ConA-inducible suppression of IgA synthesis have reported contradictory results;, In vitro IgA production after pokeweed mitogen stimulation has been variously reported as increased (Bannister et al., 1983; Egido et al., 1983; Egido et al., 1984) or normal. ConA-inducible suppression has been variously reported as decreased (Sakai et al., 1979; Egido et al., 1983) or normal (Bannister et al., 1983). And another study (Roth schild & Chatenoud, 1984) reported that only some patients displayed a decreased global, not IgA-specific, suppressor activity and suggested that IgA nephropathy is immunologically heterogeneous. Our study using T cell-T cell interaction revealed that global ConA-induced suppressor cell activity was not deficient. The lack of correlation between the numerical and functional assays, and various results may suggest that the functional assays are subject to technical variations or that patients with IgA nephropathy are heterogeneous. However, elevated helper T cell/suppressor T cell ratio and reduced suppressor T cells in patients may be related to the existence of an immunoregulatory dysfunction.

In our study, NK cell activity in patients was investigated. NK cells are nonadherent, nonphagocytic cells that express surface receptors for the Fc portion of IgG and share a variety of T lymphocyte markers.

It has been proposed that NK cells play an important role in surveillance mechanisms postulated to provide resistance toward tumor and viral infections (Herberman, 1983). Commonly, patients with IgA nephropathy have unspecified infections of upper respiratory ("synpharyngitic") or gastrointestinal tract.

Also a significant rise in antibody titer to specific infectious agents such as herpes virus, influenza, mycoplasma and gut flora was found (Woodroffe et al., 1980). Therefore, the decreased NK cell activity in patients may be related to recurrent viral infections.

However, it is not clear from these studies whether these defects are primary or secondary phenomena. Some reports (Sakai et al., 1979; Egido et al., 1983), suggested that T cell alterations in patients might be a secondary phenomenon.

As T cells are influenced by HLA-D region gene products, HLA association may support the existence of primary genetic bases for the susceptibility to IgA nephropathy. HLA associations have been described with BW, B_{12} (Richman, 1979), and most recently DR_4 (Kashiwabara et al., 1982) in patients. But this data is not as yet consistent.

REFERENCES

Bannister KM, Drew PA, Clarkson AR, Woodroffe AJ: *Immunoregulation in glomerulonephritis. Henoch-Schonlein purpura and lupus nephritis Clin Exp Immunol, 53:384, 1983.*

Basten A, Miller JPAP, Sprent J: *A receptor for antibody on B lymphocytes. I. Method for detection and functional significance. J Exp Med, 135:610, 1972.*

Berger J: *Glomerulonephrites idiopathiques a depots mesangiaux d'IGA. In: Nephrologie, edited by Hamburger J.: 2nd edition, Paris. Flammarion, 1979, pp 541-549.*

Berthoux FC, Gagne A, Sabatier JC, Ducret F, Lepetit JC, Marcelin M, Mercier B, Brizard C: *HLA-BW35 and mesangial IgA glomerulonephritis. New Engl J Med 298: 1034, 1978.*

Callard P, Feldmann G, Prandi D, Belair MF, Mandet C, Weiss Y, Druet P, Benhamou JP, Bariety J: *Immune complex type glomerulonephritis in cirrhosis of the liver. Am J Path 80:329, 1975.*

Chatenoud L, Bach M: *Abnormalities of T-cell subsets in glomerulonephritis and systemic lupus erythematosus. Kidney Int 20:267, 1981.*

Clarkson AR, Woodroffe AJ, Bannister KM, Lomax-Smith JD, Aarons I: *The syndrome of IgA nephropathy. Clin Neph 21:7, 1984.*

Egido J, Blasco R, Lozano L, Sancho J, Garcia-Hoyo R: *Immunological abnormalities in the tonsils of patients with IgA nephropathy. Clin Exp Immunol 57:101, 1984.*

Egido J, Blasco R, Sancho J, Lozano L, Gutierrez-Millet V: *Immunological studies in a familial IgA nephropathy. Clin Exp Immunol 54:532, 1983.*

Herberman RB: *Immune surveillance hypothesis; Updated formulation and possible effector mechanisms. In: Yamamura Y. ed. Progress in immunology V. New York: Academic Press, 1983: 1157.*

Kashiwabara H, Shishioo H, Tomura S, Tuchida H, Miyajima T: *Strong association between IgA nepnropathy and HLA-DR_4 antigen. Kidney Int 22:277, 1982.*

Lawrence DA, Weigle WO, Speigelberg HL: *Im-*

munoglobulins cytophilic for human lymphocytes, monocytes and neutrophils. J Clin Invest 55:368, 1975.

Lee JW, Woo JT, Nam YH, Ihm CG, Kim MJ, Kim YH, Yang MH: *Clinical and histopathologic findings in IgA nephropathy Korean J Neph, 4:142, 1985 (abstract).*

Lee JW, Park JT, Ihm SH, Kim MJ: *Follow-up study of IgA nephropathy. Korea J Neph, 4:146, 1985 (abstract).*

Lim SD, Ha YM, Woo CS, Park SY, Yun JI: *Analysis of T-cell subsets by double rosette method in healthy Koreans, J Korean Med Assoc 23:110, 1980.*

Lortan JE, Kiepiel P, Coovadi HM, Seedat YK: *Suppressor cells assayed by numerical and functional tests in chronic renal failure. Kidney Int 22:192, 1982.*

Raska KJr, Gallimore PH: An inverse relation of the oncogenic potential of adenovirus-transformed cells and their sensitivity to Killing by syngeneic natural Killer cells. Virology 123:8, 1982.

Pichman AV: *Higher prevalence of HLA-B in patients with IgA nephropathy. Am J Intern Med 90:201, 1979.*

Roccatello D, Coppo R, Piccoli G: *IgA containing material in monocytes from patients with IgA nephropathy. Nephron 38:213, 1984.*

Rothschild E, Chatenoud L: *T cell subset modulation of immunoglobulin production in IgA nephropathy and membranous glomerulonephritis. Kidney Int 25:557, 1984.*

Sakai H. Nomoto Y. Arimori S: *Decrease of IgA-specific suppressor T cell activity in patients with IgA nephropathy. Clin, Exp Immunol., 38:243, 1979.*

Sato M, Kinugasa E, Ideura T, Koshikawa S: *Phagocytic activity of polymorphonuclear leucocytes in patients with IgA nephropathy. Clin Neph 19:166, 1983.*

Solomon LR, Rawlinson VI, Howarth S, Mallick NP: *Reticulo-endothelial function in glomerulonephritis. Nephron 3754, 1984.*

Wilton JMA.: *Suppression by IgA of IgG-mediated phagocytosis by human polymorphonuclear leucocytes. Clin Exp Immun 34:423, 1978.*

Woodroffe AJ, Gormly AA, McKenzie PE, Wootton AJ, Thompson AJ, Seymour AE, Clarkson AR: *Immunologic studies in IgA nephropathy. Kidney Int. 18:366, 1980.*

Woo JT, Son YJ, Park SH, Ihm CG, Kim MJ: *The effect of levamisole on immunologic abnormaliti in glomerulonephritis. Korean J Neph 4:152, 1985 (abstract).*