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Immunoglobulin Production by Peripheral Blood Mononuclear Cells in IgA Nephropathy Patients and their Relatives

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ABSTRACT

Immunoglobulin production by peripheral blood mononuclear cells of 27 patients of IgA nephropathy and 11 relatives was determined. In comparison with 15 healthy controls, no significant difference could be observed in both IgA nephropathy patients and relatives of the group not stimulated with PWM, but in the group stimulated with PWM a significant elevation in the production of IgA, IgG and IgM was seen in IgA nephropathy patients, while in the relatives a significant elevation in production of IgA and IgG was observed. It is speculated that immune complexes mainly IgA are the chief cause of development and progression of IgA nephropathy and that IgG and IgM are also involved. In also relatives, the presence of immunological abnormalities similar to those of IgA nephropathy patients is suggested.

Elevated serum IgA level has frequently been observed in IgA nephropathy. Increase of peripheral IgA-bearing B cells[10], depressed activity of IgA-specific suppressor T cells[12], and elevated IgA production by peripheral blood mononuclear cells (PBMC)[1,3,9,14] have been reported as the causes. Familial development of IgA nephropathy[5,8,13,15] has given rise to the speculation that some familial factors might also be involved in the foregoing immunological abnormalities. However, no consistent view has yet been established on this point. We have therefore determined the immunoglobulin production by PBMC in IgA nephropathy patients and their relatives.

MATERIALS AND METHODS

The subjects of the present study were 27 IgA nephropathy patients (14 males and 13 females) with age ranging from 16 to 48 years (30.0 ± 8.8 years). Relatives were 11 cases (4 males and 7 females) with age ranging from 20 to 81 years (48.8 ± 17.0 years), composed of 9 parents, 1 elder sister, and 1 offspring. Urinalysis was normal in all cases. As controls, 15 healthy individuals (10 males and 5 females) were employed with age ranging from 26 to 65 years (34.7 ± 3.5 years).

As for the determination method, after drawing heparinized blood, mononuclear cells were separated by Ficoll-Hypaque density centrifugation and then washed three times with phosphate buffered saline (PBS). After adjusting the cell count of mononuclear cells to 10^6/ml, they were separated into a group not stimulated with pokeweed mitogen (PWM) and a group stimulated with PWM and then cultured for seven days in RPMI containing 10% fetal calf serum. In ELISA method, Nunc immuno-plate was coated for one hr with goat anti-human IgA purified with 50% of saturated ammonium sulfate (α chain specific, Cappel), goat anti-human IgG

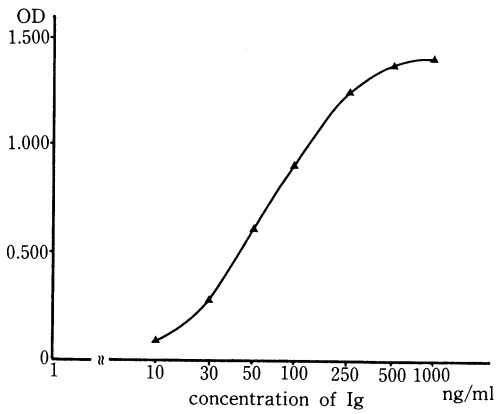

Fig. 1. Standard curve for ELISA

(Fc fragment specific, Cappel), or goat anti-human IgM (Mu chain specific, Cappel) and non-specific adsorption was blocked with 1% bovine serum albumin-PBS. To each plate was added standard solution or adequately diluted sample, that is, cultured supernatant, followed by goat anti-human Ig (heavy and light chain specific, Cappel) labeled with horse radish peroxidase. After reaction with o-phenylenediamine dihydrochloride as substrate, the reaction was stopped with 1N H_2SO_4. Their absorbance was determined at a wave length of 492 nm. Figure 1 shows the standard curve for ELISA. As liniarity was observed at 30 - 250 ng/ml, the samples were appropriately diluted so that they will fall within this range. At dilution beyond a given range, values falling outside the linear range of the standard curve were expressed as upper bound and lower bound. Statistical analysis of the data was done according to Student t-test.

RESULTS

(1) IgA production by PBMC

In the group not stimulated with PWM, amount of IgA was 157 ± 108 ng/ml in healthy controls, 148 ± 83 ng/ml in IgA nephropathy patients, and 206 ± 138 ng/ml in relatives with no significant difference observed between the three groups. In the group stimulated with PWM, amount of IgA was 165 ± 148 ng/ml in healthy controls, 399 ± 355 ng/ml in IgA nephropathy patients, and 396 ± 278 ng/ml in

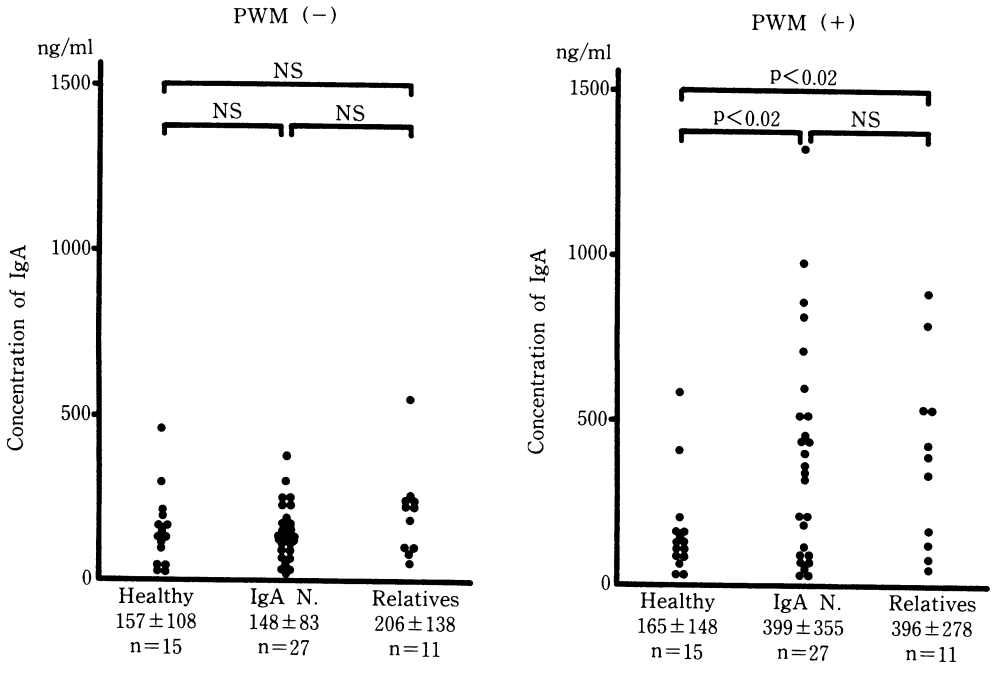

Fig. 2. *In vitro* IgA production by PBMC

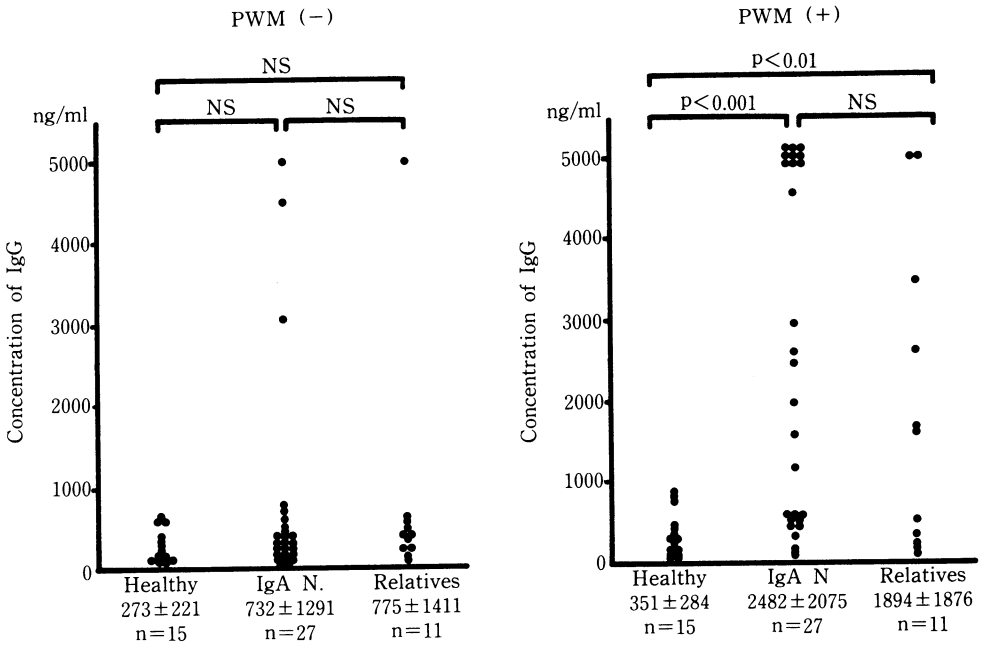

Fig. 3. *In vitro* IgG production by PBMC

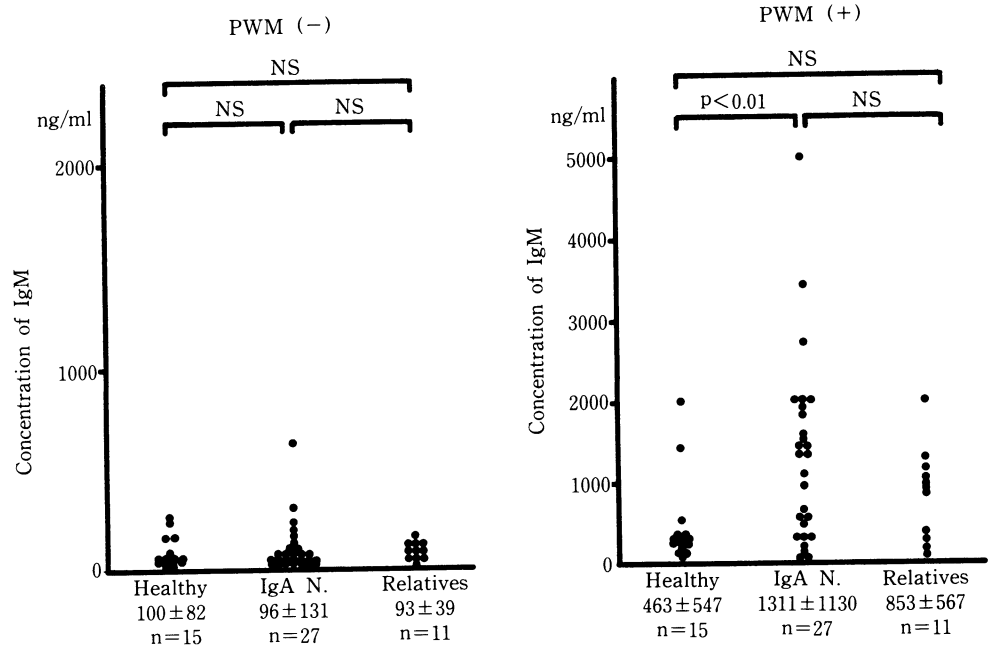

Fig. 4. *In vitro* IgM production by PBMC

relatives. The results showed that both IgA nephropathy patients and relatives had a significantly higher value than that of healthy controls ($p<0.02$) (Fig. 2).

(2) IgG production by PBMC

In the group not stimulated with PWM, amount of IgG was 273 ± 221 ng/ml in healthy controls, 732 ± 1291 ng/ml in IgA nephropathy patients, and 775 ± 1411 ng/ml in relatives with no significant difference observed between

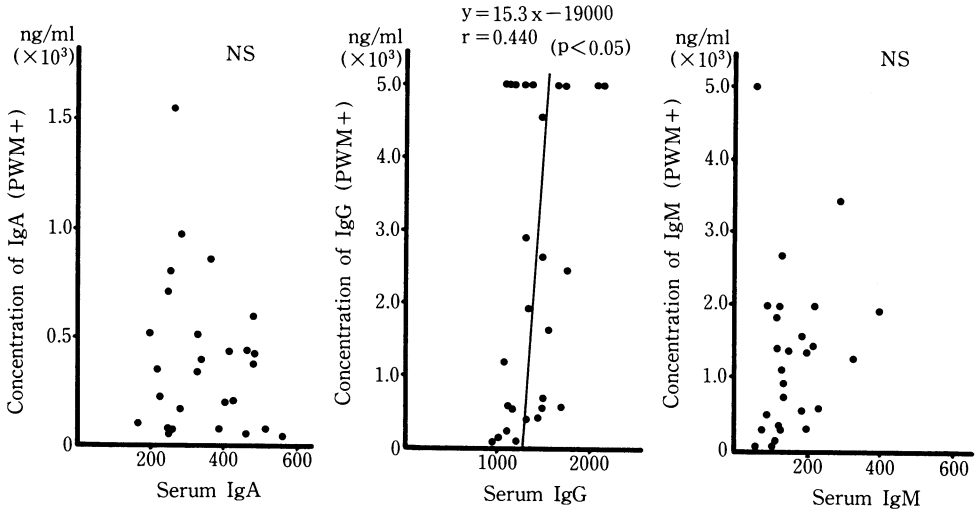

Fig. 5. Relationship between amounts of Ig production by PBMC and serum Ig levels in IgA nephropathy patients

the three groups. In the group stimulated with PWM, amount of IgG was 351 ± 284 ng/ml in healthy controls, 2482 ± 2075 ng/ml in IgA nephropathy patients, and 1894 ± 1876 ng/ml in relatives. These results show that both IgA nephropathy patients and relatives had a significantly higher value than that of healthy controls ($p<0.001$, $p<0.01$, respectively) (Fig. 3).

(3) IgM Production by PBMC

In the group not stimulated with PWM, amount of IgM was 100 ± 82 ng/ml in healthy controls, 96 ± 131 ng/ml in IgA nephropathy patients, and 93 ± 39 ng/ml in relatives with no significant difference being demonstrable between the three groups. In the group stimulated with PWM, amount of IgM was 463 ± 547 ng/ml in healthy controls, 1311 ± 1130 ng/ml in IgA nephropathy patients, and 853 ± 567 ng/ml in relatives. These results show that IgA nephropathy patients had a significantly higher value than that of healthy controls ($p<0.01$) (Fig. 4).

(4) Relationship between amounts of immunoglobulin production by PBMC and serum immunoglobulin levels

A study was made to determine whether a correlation existed between the amounts of IgA, IgG, and IgM following PWM stimulation and the levels of serum IgA, IgG, and IgM. The results showed that there was no correlation in IgA and IgM, but a significant correlation was observed in IgG ($p<0.05$) (Fig. 5).

DISCUSSION

In recent years reports have been made on immunological abnormalities in IgA nephropathy such as elevated serum IgA level, increase of IgA-bearing B cells[10], increase of IgA-specific helper T cells[6,7,14], depressed activity of IgA-specific suppressor T cells[12], increase of helper T cells/suppressor T cells in T cell subsets[2,4,11], and elevated production of immunoglobulins by PBMC[1,3,9,14], but it cannot be said that the views are consistent. The authors have therefore made a study on 27 patients of IgA nephropathy with determinations on immunoglobulin production by PBMC as one of the indices of the foregoing immunological functions. The results showed in IgA nephropathy patients that the production of IgA, IgG, and IgM was significantly elevated in PWM stimulated group when compared to the age matched control group. Several reports have been published on immunoglobulin production by PBMC. According to the report of Egido et al[2], IgA nephropathy patients did not show any significant difference from healthy controls in the group not stimulated with PWM, but in the group stimulated with PWM, IgA production was significantly elevated. On the other hand, Cosio et al[1] have reported that in comparison with healthy controls, the production of IgA, IgG, and IgM was elevated in IgA nephropathy patients in the group not stimulated with PWM, but in the group stimulated with PWM no significant difference between the two

could be demonstrated and that among IgA nephropathy patients the production of IgG and IgM was elevated only in severe cases. Furthermore, according to Linne et al[9], a tendency was observed for the production of IgA, IgG, and IgM to be more elevated in IgA nephropathy patients than healthy controls of both the group not stimulated and the group stimulated with PWM, but the difference was not statistically significant. Also, Sakai et al[14] have reported that IgA production was elevated more in the IgA nephropathy patients than in healthy controls of the group not stimulated with PWM. Though some differences can be observed between our results and those reported by various workers, race, disease stage, and PWM titer may be involved in these differences. However, it is speculated that immune complexes mainly IgA are the chief causes of development and progression of IgA nephropathy and that IgG and IgM are also involved.

Furthermore, studies were also made on relatives. The results showed that in comparison with healthy controls the production of IgA and IgG was significantly elevated in the group stimulated with PWM. However, the age of the relatives of the present study ranged from 20 to 81 years (48.8 ± 17.0 years) which is statistically significantly higher than that of the healthy controls and the factor of aging cannot be completely denied. Therefore, the relatives were divided into three cases of the younger group of age 20 - 40 years (31.7 ± 10.4 years) with no significant difference in age with the healthy controls and eight cases of the older age group of age 41 - 81 years (55.3 ± 14.4 years), but this study did not reveal any significant difference in the amounts of IgA and IgG production. It is therefore considered that the factor of age can be excluded from our relatives. This suggests that the relatives also show immunological abnormalities resembling those of IgA nephropathy patients. It is speculated that there is a genetic involvement in the development and progression of IgA nephropathy or a common exposure to some antigenic stimulation after birth.

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