Glomerular and Serum IgG Subclasses in Diffuse Proliferative Lupus Nephritis, Membranous Lupus Nephritis, and Idiopathic Membranous Nephropathy

Aki KUROKI, Takanori SHIBATA, Hirokazu HONDA, Daisuke TOTSUKA, Kenji KOBAYASHI and Tetsuzo SUGISAKI

Abstract

Objective To determine whether a different renal histopathology is associated with the characteristic IgG subclass distribution, and whether a distinct IgG subclass distribution is involved in a unique immunopathological expression, we compared the distributions of glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis (DPLN), membranous LN (MLN), and idiopathic membranous nephropathy (MN).

Patients and Methods The glomerular IgG subclass distributions in patients with DPLN (n=7), MLN (n=10) or MN (n=16) were assessed by direct immunofluorescence microscopy. Serum levels of each IgG subclass were quantitated by ELISA in DPLN, MLN, and MN patients, and in normal human sera (NHS) (n=14).

Results IgG1, IgG2, IgG3, and, to a lesser degree, IgG4 were similarly present in glomerular deposits in both DPLN and MLN. In contrast, IgG4 was the predominant glomerular IgG subclass in MN. Regarding the serum IgG subclasses, the mean IgG subclass concentrations and the mean proportion of each IgG subclass to the total IgG (%IgG subclass) in DPLN and MLN were not significantly different from those in NHS, except for the increased %IgG1 in MLN. In MN, the mean %IgG4 was selectively increased ($p<0.01$ vs NHS) in association with a slightly elevated IgG4 concentration; however, the mean concentrations of other IgG subclasses were significantly decreased ($p<0.01$ vs NHS), and the %IgG subclasses were not significantly different from those in NHS.

Conclusions The results indicate that the IgG subclass distribution is not associated with the different renal histopathologies of DPLN and MLN. This study also shows the selective significance of IgG4 in MN, but not in MLN, another form of membranous glomerulopathy. (Internal Medicine 41: 936–942, 2002)

Key words: systemic lupus erythematosus, membranous

glomerulopathy, IgG4, renal immunopathology

Introduction

Human IgG molecules are classified into four different IgG subclasses according to the immunologically distinct constant region of the heavy chain. Accordingly, different IgG subclasses have distinct physicochemical and biological properties (1). Apparently, the IgG class of antibodies is the most important Ig isotype in immunopathogenesis for both lupus nephritis (LN) and idiopathic membranous nephropathy (MN), because they unequivocally have glomerular IgG deposits (2).

Using an animal model, experimental studies on the pathogenic significance of murine IgG3 in autoimmune MRL/MpJ-*lpr/lpr* mice (3) suggest that a particular IgG subclass may be operative in the development of an immune-complex (IC) type of glomerulonephritis (ICGN) in man. Several investigators determined by immunofluorescence microscopy (IF) the deposition of IgG subclasses in patients with ICGN, such as LN, MN and membranoproliferative glomerulonephritis (4–9). However, the glomerular IgG subclass distribution in membranous lupus nephritis (MLN), which is characterized by diffuse thickening of glomerular capillary walls, is still controversial (5, 8). In addition, it is uncertain whether an essential IgG subclass exists in membranous glomerulopathy, such as MN and MLN. Furthermore, the significance of serum IgG subclass levels in MN remains unclear.

In the present study, to determine whether a different renal histopathology is associated with the characteristic IgG subclass distribution, and whether a distinct IgG subclass distribution is involved in the unique immunopathological expression, we compared the distributions of glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis (DPLN), which is the most severe type of proliferative LN, MLN, and MN. Our results indicate that the IgG subclass distribution is not associated with the different histopathological expressions

From the Department of Nephrology, Showa University School of Medicine, Tokyo

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Reprint requests should be addressed to Dr. Takanori Shibata, the Department of Nephrology, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666

of DPLN and MLN, and suggest that IgG4 is closely related to MN, which is not applicable to another form of membranous glomerulopathy, MLN. We will also discuss the role of IgG4 in the development of MN.

Patients and Methods

Patients

Renal biopsy specimens were obtained from 17 patients with systemic lupus erythematosus (SLE) and 16 patients with MN who were diagnosed based on the clinical and renal biopsy findings in our department. All the patients with SLE fulfilled the 1982 revised criteria of the American Rheumatism Association (10) for establishing a diagnosis of SLE. Renal biopsy specimens were divided and processed for light, immunofluorescence and electron microscopies. For IF, renal tissues were snap frozen in liquid nitrogen and cut into 4 μ m sections. The glomerular deposits of IgG, IgM, IgA, C1q and C3 in the biopsy specimens were examined by routine IF and significant glomerular IgG deposits in LN and MN were substantiated. The histological classification of LN was performed according to the World Health Organization morphologic classification (2), by which seven patients with DPLN (class IV) and 10 patients with MLN (class V) were enrolled in the present study. In the DPLN group, five patients were classified as class IV_A, one patient as class IV_B, and one patient as class IV_D. In the MLN group, three patients were classified as class V_A, and seven patients as class V_B. The histological stage was examined in 15 of 16 patients with MN who were then classified into four stages according to the electron microscopic findings (2). Ten patients were classified as stage II, one patient as stage II-III, and four patients as stage III. All of the patients with SLE presented active renal manifestations with hypocomplementemia. The patients with MN had no systemic diseases that are possibly related to the development of membranous nephropathy.

Serum samples

Serum samples were obtained from the patients with LN or MN, near the time of renal biopsy. Fourteen normal human serum (NHS) samples were obtained from healthy volunteers. Blood samples were allowed to clot at 37°C for 30 minutes to avoid cryoprecipitation, then the separated sera were frozen at -20°C until use.

Polyclonal antibodies

FITC-conjugated goat anti-human IgG, IgM, IgA, C1q and C3 specific antibodies, and the purified IgG fraction of goat anti-human IgM specific antibodies were purchased from Cappel Laboratories (West Chester, PA, USA). The alkaline-phosphatase (AP)-conjugated mouse anti-human IgG specific antibodies were purchased from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA, USA).

Monoclonal antibodies (mAbs)

The FITC-conjugated mouse anti-human IgG subclass specific mAbs used were as follows: clone Nos. HP-6069 (ZYMED

Laboratories, San Francisco, CA, USA; anti-IgG1), HP-6014 (Sigma Chemical Co., St. Louis, Mo, USA; anti-IgG2), HP-6047 (ZYMED Laboratories; anti-IgG3), and HP-6025 (Sigma Chemical Co.; anti-IgG4). All of the antibodies were of the mouse IgG1 subclass. The purified IgG fraction of mouse anti-human IgG subclass specific mAbs (Calbiochem Corp., La Jolla, CA, USA) used were HP6069, HP6002 (anti-IgG2), HP6047 and HP6025. HP6002 was also of the mouse IgG1 subclass. These anti-human IgG subclass specific mouse mAbs were extensively evaluated and identified as the most suitable mAbs available in terms of reactivity and specificity (11, 12, reviewed in 13). The AP-conjugated mouse anti-human IgM mAb used was clone No. HP-6083 (ZYMED Laboratories). The purified mouse anti-human IgE mAb used was clone No. MCA E-01-P (Yamasa Shoyu Co., Ltd., Choshi, Chiba) and the peroxidase-conjugated mouse anti-human IgE mAb used was clone No. MCA E-09-HRP (Yamasa Shoyu Co., Ltd.).

Human IgG myeloma proteins

Purified human IgG1 (No.I-3889), IgG2 (No.I-4139), IgG3 (No.I-4389), and IgG4 (No.I-4639) myeloma proteins purchased from Sigma Chemical Co. were used to verify the reactivities and specificities of the human IgG subclass specific mouse mAbs under the assay condition employed in our ELISA of IgG subclasses.

Glomerular IgG subclasses

Glomerular IgG subclass deposition was examined by direct IF using FITC-conjugated mouse anti-human IgG subclass specific mAbs. The fluorescence intensity of glomerular staining was graded as follows: negative, 0; very weak, 0.5; weak, 1; moderate, 2; and strong, 3, and scored for each biopsy specimen. The results are expressed as the frequency of positive biopsies (percentage) and the intensity of fluorescence (mean score) for each IgG subclass.

ELISA of IgG subclasses and IgM

IgG subclass levels in sera were quantitated by IgG subclass specific sandwich ELISA as described by Hamilton (13) with minor modifications, according to an ELISA procedure published previously (14). Briefly, microtiter plates (Nunc, Roskilde, Denmark) were coated with one of the purified IgG fractions of human IgG subclass specific mouse mAbs at 5 μ g/ml for HP6069, HP6002 and HP6025, and at 2.5 μ g/ml for HP6047 as capture antibodies. Then, appropriately diluted serum samples were added to microtiter wells. The assays were developed with AP-conjugated mouse polyclonal anti-human IgG specific antibodies as detection antibodies. The reactivities and specificities of the ELISA of IgG subclasses were substantiated by constructing dose-response curves for serial dilutions (two fold) of four different IgG subclasses of the human myeloma protein, ranging from 1,000 ng/ml to 2 ng/ml. When the O.D. value of an appropriate corresponding subclass was approximately 1.70 at 500 ng/ml, those of irrelevant subclasses were from 0.11 to 0.19 (background O.D. values: 0.08–0.11). In each assay, the standard curve was established using the

human standard serum, NOR-01/91 (Nordic Immunological Laboratories, Tilburg, The Netherlands) for quantification of each IgG subclass. When dilution curves were obtained using the standard serum, the minimal detectable levels of IgG1, IgG2, IgG3 and IgG4 were approximately 20, 40, 8, and 10 ng/ml, respectively. The sum of each IgG subclass level was defined as the total IgG. The serum IgG subclass levels are expressed as the concentration of each IgG subclass, and the proportion of each IgG subclass to the total IgG (%IgG subclass). For quantification of IgM levels in sera, microtiter plates were coated with goat anti-human IgM at 5 µg/ml. Diluted serum samples were added to microtiter wells. Bound IgM was detected with AP-conjugated HP-6083. The standard curve was established using purified polyclonal human IgM (Calbiochem Corp.).

IgE ELISA

For quantification of IgE levels in sera, microtiter plates were coated with mouse anti-human IgE mAb at 5 µg/ml. Diluted sera were added to microtiter wells. The assays were developed with peroxidase-conjugated mouse anti-human IgE mAb, according to the manufacturer's instructions. *O*-Phenylenediamine (OPD) dihydrochloride (Sigma Chemical Co.) at 0.4 mg/ml in 0.05 M phosphate-citrate buffer (pH 5.0), containing 0.012% H₂O₂ was used as the substrate. The reaction was terminated with 200 µl of 2 M H₂SO₄. Then, ODs were read at 492 nm. The standard curve was established using a purified human IgE myeloma protein (Yamasa Shoyu Co., Ltd.).

Statistical analysis

Statistical analysis was performed with the Mann-Whitney U test. Probability values greater than 5% were considered insignificant.

Results

Patient characteristics

Clinical parameters of the DPLN (7 female), MLN (10 female) and MN (11 male and 5 female) patients are shown in Table 1. The mean ages for the group of patients with DPLN, MLN, or MN were 36.3±12.3 (mean±SD; range, 18–49), 39.1±13.3 (range, 19–52), and 54.9±14.1 (range, 24–76) years,

respectively ($p < 0.01$, MN vs DPLN, MLN). A significant level of urine protein and decreased levels of mean serum total protein and albumin were observed in these three groups of patients. There were no significant differences in levels of urine protein, serum total protein, serum albumin and serum creatinine in the three groups of patients.

Glomerular IgG subclass distribution in DPLN, MLN and MN patients

Glomerular IgG subclass distributions in the DPLN, MLN and MN groups are shown in Fig. 1. In the DPLN group, there was no significant difference in the frequency of each IgG subclass deposition. Regarding the mean score of each IgG subclass deposition, that of IgG4 deposition (0.36 ± 0.38 , mean±SD) was significantly lower than those of IgG2 (1.29 ± 0.70) and IgG3 (1.00 ± 0.58) in the DPLN group (Fig. 1A). In the MLN group, IgG2 deposition frequency was significantly high (100%) compared with IgG3 (60%) and IgG4 (60%), and the mean score of IgG4 deposition (0.50 ± 0.62) was lower than those of IgG1 (0.85 ± 0.34) and IgG2 (1.30 ± 0.48) (Fig. 1B). Overall, IgG1, IgG2 and IgG3, and, to a lesser degree, IgG4, were similarly present in glomerular deposits in both DPLN and MLN groups. In the MN group, IgG4 and IgG1 were the dominant glomerular IgG subclasses, because IgG4 was present in 100% of the biopsies and IgG1 in 88%. On the other hand, both IgG2 and IgG3 were present in 25% of the biopsies studied. Regarding the mean score of each IgG subclass, a strikingly high mean score of IgG4 deposition (1.94 ± 0.85) and a significant, but weak IgG1 deposition (0.72 ± 0.36) were noted. In contrast, the mean scores of IgG2 and IgG3 depositions were very low (0.16 ± 0.30) (Fig. 1C). Examples of immunofluorescence staining of glomerular IgG subclass deposits in DPLN and MN are shown in Figs. 2 and 3, respectively.

Comparison of glomerular IgG subclass deposition in DPLN, MLN and MN patients

When the deposition of each glomerular IgG subclass was compared between the DPLN and MLN groups (Figs. 1A and 1B), there was no significant difference in the deposition frequency or the intensity of immunofluorescence staining. However, when the deposition of each glomerular IgG subclass was compared between the MLN and MN groups (Figs. 1B and

Table 1. Subject Characteristics

Diagnosis	N	Sex (M/F)	Age (yr)	UP (g/day)	T-P (g/dl)	Alb (g/dl)	Cr (mg/dl)
DPLN	7	0/7	36.3±12.3 ^a	3.9±2.0	5.1±0.9	2.5±0.5	1.0±0.2
MLN	10	0/10	39.1±13.3	2.6±2.4	5.8±1.0	2.8±0.9	1.1±0.6
MN	16	11/5	54.9±14.1*	3.6±2.9	5.0±0.7	2.7±0.6	1.0±0.2

DPLN: diffuse proliferative lupus nephritis, MLN: membranous lupus nephritis, MN: idiopathic membranous nephropathy, UP: urinary protein, T-P: serum total protein, Alb: serum albumin, Cr: serum creatinine. ^a Values indicate means±SD. * $p < 0.01$ (vs DPLN, MLN).

Figure 1. Glomerular IgG subclass distribution in diffuse proliferative lupus nephritis (DPLN), membranous lupus nephritis (MLN) and idiopathic membranous nephropathy (MN). Glomerular IgG subclass deposition was examined by direct immunofluorescence microscopy, and graded on a 0-to-3 scale based on the intensity of fluorescence. A, DPLN (n=7); B, MLN (n=10); C, MN (n=16). *Left*, Frequency of IgG subclass deposition. Results are expressed as the percentage of positive biopsies that represent the score ≥ 0.5 for one given IgG subclass. *Right*, Intensity of IgG subclass deposition. Results are expressed as the score. Each column and each bar represent mean+SD. Both positive and negative cases are included in the calculation of the mean score. The significant difference is shown as * $p < 0.05$ and ** $p < 0.01$.

1C), there were significant differences in both the deposition frequencies and mean scores of IgG2 and IgG4 depositions. That is, IgG2 staining, MLN vs MN, $p < 0.01$; and IgG4 stain-

Figure 2. Glomerular IgG subclass deposits in a patient with diffuse proliferative lupus nephritis determined by immunofluorescence microscopy ($\times 100$). IgG subclass deposition was examined using FITC-conjugated mouse anti-human IgG subclass specific mAbs. A, IgG1 (score 1); B, IgG2 (score 2); C, IgG3 (score 1); D, IgG4 (score 0.5).

Figure 3. Glomerular IgG subclass deposits in a patient with idiopathic membranous nephropathy determined by immunofluorescence microscopy ($\times 100$). IgG subclass deposition was examined using FITC-conjugated mouse anti-human IgG subclass specific mAbs. A, IgG1 (score 0.5); B, IgG4 (score 2). Both IgG2 and IgG3 were negative in this case.

ing, MLN vs MN, $p < 0.01$. These data indicate that IgG4 is the predominant IgG subclass in MN, but not in MLN.

Serum IgG subclass levels in DPLN, MLN and MN patients

The levels of serum IgG subclasses in the DPLN, MLN and MN groups are shown in Table 2. In DPLN and MLN groups although it seemed that the mean serum concentrations of the IgG subclasses, especially that of IgG1 increased compar-

Table 2. Serum IgG Subclass Levels in DPLN, MLN, and MN Patients

Group	N	IgG subclass concentration (mg/ml)				Total IgG (mg/ml)	% IgG subclass			
		IgG1	IgG2	IgG3	IgG4		IgG1	IgG2	IgG3	IgG4
DPLN	7	8.08±3.57*	4.56±2.38	0.48±0.20	0.52±0.48	13.65±5.69	59.1±7.5	33.2±9.2	3.7±1.2	3.9±3.3
MLN	10	15.77±12.21	4.41±1.76	0.58±0.76	0.75±0.77	21.51±14.21	68.2±11.5*	26.3±11.7	2.3±1.6	3.2±2.0
MN	16	3.68±1.31**	2.84±2.26*	0.24±0.15**	0.69±0.83	7.43±3.88**	52.7±10.8	35.9±11.1	3.3±2.2	8.1±6.4**
NHS	14	6.42±2.08	4.11±1.76	0.44±0.21	0.40±0.42	11.37±2.09	56.4±13.5	32.2±13.0	4.0±1.9	3.4±2.9

DPLN: diffuse proliferative lupus nephritis, MLN: membranous lupus nephritis, MN: idiopathic membranous nephropathy, NHS: normal human sera, % IgG subclass: proportion of each IgG subclass to total IgG. *Values indicate means±SD. * $p<0.05$ (vs NHS), ** $p<0.01$ (vs NHS).

Table 3. Serum IgM and IgE Levels in DPLN, MLN, and MN Patients

Group	N	IgM (mg/ml)	IgE (μg/ml)
DPLN	7	0.97±0.50*	0.72±0.66
MLN	10	1.07±0.77	2.08±3.15*
MN	16	1.08±0.76	0.50±0.77
NHS	14	1.22±0.61	0.17±0.21

DPLN: diffuse proliferative lupus nephritis, MLN: membranous lupus nephritis, MN: idiopathic membranous nephropathy, NHS: normal human sera. *Values indicate means±SD. * $p<0.05$ (vs NHS).

with those in NHS, their differences were statistically insignificant. Regarding the mean %IgG subclasses, they were not significantly different from that in NHS, except for the increased %IgG1 in the MLN group. Notably, there were no significant differences in the levels of serum IgG subclasses between the DPLN and MLN groups. In MN group, the mean serum concentrations of IgG1, IgG2 and IgG3 were significantly lower than those in the NHS. In contrast, the serum IgG4 concentration was elevated, although the elevation was statistically insignificant. When the mean %IgG subclasses were compared with that in NHS, the %IgG1, %IgG2, and %IgG3 were almost comparable. However, the %IgG4 significantly increased in the MN group. When the serum levels of total IgG in the three groups of patients were compared with that in NHS, a significant difference was observed in only the MN group.

Serum IgM and IgE levels in DPLN, MLN and MN patients

The levels of serum IgM and IgE in the DPLN, MLN and MN groups are shown in Table 3. In the DPLN, MLN and MN groups, serum IgM levels were not significantly different from that in NHS. Although serum IgE levels were elevated in the three groups of patients, a significantly higher value was observed in only the MLN group ($p<0.05$, vs NHS).

Discussion

By comparing the glomerular IgG subclass distribution in the three groups of patients with DPLN, MLN, or MN, we have shown that IgG4 is the distinctive glomerular IgG subclass in the MN group. In contrast, predominant deposition of IgG4 was found in neither the MLN nor DPLN group, but rather the staining intensity of IgG4 deposition was less than those of other IgG subclasses. Furthermore, we have shown that serum IgG4 levels were selectively elevated in patients with MN. Thus, the predominant deposition of IgG4 along the glomerular capillary wall and the elevated serum IgG4 levels in patients with MN support the distinctive significance of IgG4 in the immunopathogenesis of MN.

In the present patients with MN, the data on the glomerular IgG subclass distribution obtained by IF are almost in agreement with previous reports (6, 7), but at variance in the positive frequency or staining intensity of each IgG subclass. A significant but weak deposition of IgG1 in association with remarkable IgG4 deposition was noted in the MN group. Regarding the glomerular IgG subclasses in LN, although diverse reports were published, comparative study of the glomerular IgG subclass deposition between DPLN and MLN was not performed, except for the study by Roberts et al (5). They showed the predominant deposition of IgG1 and IgG3 in DPLN patients, but that of IgG4 in MLN patients. Moreover, they suggested that the distinct IgG subclass distribution might be responsible for the unique histopathology *via* different complement activating capabilities of glomerular IgG subclasses. However, our data contradict this hypothesis, because IgG1, IgG2 and IgG3 were significantly distributed in both the DPLN and MLN groups. Accordingly, the distinct histopathologic expressions of DPLN and MLN could not be explained by the glomerular IgG subclass distribution. In this regard, Haas noted that all the IgG subclasses were present in patients with MLN, but the staining intensity of IgG3 exceeded that of IgG4 (8). Possibly, such different results may be due to either the difference in reagents used, such as FITC-conjugated antibodies

applied in IF, or difference in populations of patients. The present results of the comparative study of the glomerular IgG subclass deposition between MLN and MN indicate that IgG4 is the predominant glomerular IgG subclass in MN, but not in another form of membranous glomerulopathy, MLN. This finding agrees with that of Haas (8).

The most notable observation in the present study was the significant increase in the %IgG4 associated with a slightly elevated serum IgG4 concentration in patients with MN, although the serum concentrations of the other three IgG subclasses decreased. This indicates that IgG4 production but not that of other IgG subclasses may be significantly enhanced, and that would be related to glomerular deposition of IgG4, in combination with still undetermined nephritogenic antigen(s) in MN. Thus, it can be proposed that IgG4 is related to the immunopathogenesis of MN, even if the extent of its serum concentration elevation is relatively low. Although it seemed that the IgG subclass levels increased in the sera from DPLN and MLN patients, the mean concentration of each IgG subclass and the %IgG subclass were not significantly different from those in NHS, except for the increased %IgG1 in MLN. In addition, the serum IgE levels were significantly increased only in patients with MLN. These results may reflect in part the overall activation of B cells in SLE. However, the significance of the levels of serum IgG subclasses in LN is still unclear.

One of the factors that may influence the serum IgG subclass levels in patients with glomerular disease who develop either nephrotic syndrome or a moderate degree of proteinuria, is urinary excretion of IgG subclasses. In addition, the depression of the serum IgG levels in patients with nephrotic syndrome due to a primary glomerulopathy, such as minimal change nephrotic syndrome (MCNS) and MN may be considered (15–17). However, since IgG subclasses appear in the urine in proportions similar to their relative concentrations in the serum (15), the possibility of selective urinary loss of particular IgG subclasses can be excluded. Furthermore, the report that the synthesis of IgG by pokeweed mitogen-stimulated peripheral blood lymphocytes is diminished in MN and MCNS *in vitro* (16, 17) supports the idea that the variable levels of serum IgG subclasses in patients with LN or MN are determined by the difference in the production rate of each IgG subclass. In this context, the increase in the %IgG4 in association with a slightly elevated serum IgG4 concentration, while the total serum IgG level is decreased in patients with MN, is significant.

In the present study, we have shown that levels of serum IgG4 are elevated in patients with MN. Other investigators reported conflicting results, in which they described that neither serum IgG4 concentration nor %IgG4 is increased (7, 9). This discrepancy may be partly ascribed to either the ELISA methodology employed, or the difference in patients' profile, such as the clinical course, because MN is subject to fluctuations in disease activity.

MN is an ICGN induced by either circulating ICs or *in situ* IC formation or both pathogeneses (18). How is IgG4 involved in the subepithelial immune-deposit formation in MN? Several

hypothetical roles of IgG4 could be discussed. First, the size of IgG4-containing IC may be small, which makes it easy to be localized along the capillary wall, since it has been reported that IgG4 antibodies form small, nonprecipitating ICs (19). Second, the affinity of IgG4 may be low in MN, because ICs tend to be smaller and localize in subepithelial spaces when low-affinity antibodies are produced (18). A previous study indicated that the level of IgG4 CIC composed of low-avidity antibodies was increased in 10 of 35 patients with MN (20). Third, the charge of IgG4 or IgG4-containing ICs may be critical, because the capillary wall would be more accessible to cationically charged molecules *via* charge interactions (18). In this respect, as the charge of IgG4 is more anionic than those of other IgG subclasses (13), the charge of the corresponding antigen(s) of IgG4 would be cationic in MN, which facilitates its localization along the glomerular basement membrane, leading to subepithelial immune-deposit formation by complexing with circulating IgG4 *in situ*. Alternatively, it is of special interest to note a recent proposal of Zack et al regarding the unique characteristic of IgG4, that is, its constant region possesses IgG Fc-binding reactivities (21). If serum IgG4 in MN represents such immunochemical property, this may be responsible for a novel mechanism of IC formation *in situ*. This issue is under investigation in our laboratory. Thus, although the nature of IgG4 needs to be determined in the future, the IgG4 molecule in MN may have unique immunological properties that are related to the subepithelial immune-deposit formation.

Production of IgG1 and IgG3, or IgG2 antibodies is stimulated by protein antigens or carbohydrate antigens, respectively (1, 13). Regarding IgG4, it has been reported that prolonged antigenic stimulation results in IgG4 antibody production (22). Accordingly, it is very important to determine antigenic stimuli that induce IgG4 production in MN. At present, the specificities of IgG4 and the mechanisms of IgG4 production in MN remain unclear. In recent years, human IgG subclass production and switch mechanisms have been elucidated in part on the basis of the role of Th cell-derived cytokines (23–25). The increase in %IgG4 in MN sera may indicate the involvement of Th2 cytokines, such as IL-4, although the increase is not accompanied by significant elevation of IgE levels. Interestingly, Jeannin et al recently reported the role of IL-10 in the differential production of IgG4 and IgE *in vitro* (26). Therefore, certain cytokines may participate in the selective production of IgG4 in MN. Further studies on the role of IgG4 in the pathogenesis of MN may provide important information that may lead to the development of therapeutic approaches for MN.

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