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Distribution of IgG subclasses in membranous nephropathy

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SUMMARY

The distribution of human IgG subclasses among the glomerular deposits in human membranous nephropathy was examined by immunofluorescence with subclass specific monoclonal antibodies. Large amounts of granular deposits of IgG4 were identified along the capillary loops in all 12 patients, and seven patients had small amounts of IgG1 deposits. Neither IgG2 nor IgG3 deposits were detectable in any of the patients. On the contrary, all four IgG subclasses were detected in membranoproliferative glomerulonephritis and lupus nephritis with a predominance of IgG1 and IgG3. The results indicate that IgG4 is predominant in the glomerular deposits in membranous nephropathy and may play an important role in its pathogenesis.

Keywords IgG4 IgG subclasses membranous nephropathy

INTRODUCTION

Most forms of human glomerulonephritis (GN) are thought to be immunologically mediated on the basis of deposits of immunoglobulins and complement components observed by immunofluorescence in the affected glomeruli (McCluskey *et al.*, 1966; Dixon, 1968). Specific patterns of IgG deposits are seen in various types of GN. Membranous nephropathy (MN) is characterized by subepithelial IgG deposits, but the pathogenesis of this disorder is still unclear. Human IgG consists of four subclasses (IgG1, IgG2, IgG3 and IgG4) that can be recognized by antigenic differences in their heavy chains (Kunkel *et al.*, 1964; Grey & Kunkel, 1964; Kunkel, Yount & Litwin, 1966). Each subclass has different biological activities, and the IgG subclasses may be preferentially produced in response to different antigens (Spiegelberg, 1974). Thus, examination of the distribution patterns of IgG subclasses in glomerular deposits in different types of nephritis may provide insight into the immunological processes involved in GN. Polyclonal antisera have been used to examine the distribution of IgG subclasses in human glomerular lesions with varied results (Lewis, Busch & Schur, 1970; Bannister *et al.*, 1983; McPhaul & Dixon, 1971; Puritz *et al.*, 1973; Roberts *et al.*, 1983).

In this study, mouse monoclonal antibodies (MoAb) specific for each of the four human IgG subclasses were employed to examine the distributions of IgG subclasses in glomerular deposits of MN and in other forms of GN. IgG4 was found to be the predominant subclass in IgG deposits in MN, whereas IgG1 and IgG3 were the prevalent IgG subclasses in lupus nephritis.

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MATERIALS AND METHODS

Patients. Twelve patients with MN were examined. In addition, renal biopsy specimens were studied from two patients with membranoproliferative GN (MPGN), eight with lupus nephritis (SLE) and five with no detectable glomerular deposits (controls). The type of GN in these patients was defined according to clinical and histological criteria, including electron microscopic examinations (Churg & Sobin, 1982).

Antibodies. Fluorescein conjugated antisera specific for human γ-chains, α-chains, μ-chains, Clq and C3c were purchased from Behringwerke (FRG) and used for immunofluorescence analyses. Their specificities were confirmed by double immunodiffusion, immunoelectrophoresis and immunofluorescence. Mouse hybridoma antibodies specific for each of the four human IgG subclasses (NL-16, GOM-2, C3-8-34 and 9441-SA) were used to examine IgG subclass distributions. The specificities of NL-16 (anti-IgG1 antibodies; mouse Ig isotype, γ_1 κ), GOM-2 (anti-IgG2 antibodies; mouse Ig isotype, γ_1 κ), C3-8-34 (anti-IgG3 antibodies; mouse Ig isotype, γ_{2a}, κ), and 9441-SA (anti-IgG4 antibodies; mouse Ig isotype, γ_1 κ) have been described elsewhere (Mayumi *et al.*, 1983; Low *et al.*, 1982). Fluorescein or rhodamine labelled affinity purified goat antibodies to mouse immunoglobulins, which had been extensively absorbed with human immunoglobulins and did not cross-react with human immunoglobulins by immunofluorescence, were used to detect the mouse MoAb by indirect immunofluorescence.

Immunofluorescence analyses of glomerular deposits. Biopsy specimens obtained from the patients described above were frozen immediately with acetone–dry ice at the time of renal biopsy and kept at -70°C until use. Two micrometre sections were obtained in a cryostat and fixed with acetone for 5 min. Analyses for glomerular deposits of IgG, IgA, IgM, Clq and C3c were performed by direct immunofluorescence using the antisera described above at appropriate concentrations for immunofluorescence. The biopsy specimens were incubated with 10 μl of the diluted antisera for 30 min at room temperature. IgG subclass distributions were examined by indirect immunofluorescence using the mouse MoAb and the fluorescein or rhodamine labelled goat anti-mouse immunoglobulin antibodies described above according to methods described elsewhere (Mayumi *et al.*, 1983). Briefly, biopsy specimens were first incubated either with 10 μl of affinity purified anti-human IgG1 antibody at a concentration of 0·1 mg/ml or each of the other three anti-human IgG subclass MoAb in ascitic fluid diluted to a titre optimal for immunofluorescence for 30 min at room temperature. After washing, the specimens were then incubated with fluorescein or rhodamine labelled goat antibodies to mouse immunoglobulin at a concentration of 0·1 mg/ml for

Table 1. Distribution of immunoglobulins and complement components of patients with MN

Case number	IgG	IgM	IgA	IgG1	IgG2	IgG3	IgG4	Clq	C3c
1	+++	−	−	+	−	−	+++	−	−
2	+++	−	−	+	−	−	+++	−	−
3	++	−	−	+	−	−	++	−	−
4	+++	+	−	++	−	−	+++	+	−
5	+++	+	−	+	−	−	+++	+	+
6	++	+	−	−	−	−	++	−	+
7	++	−	−	−	−	−	++	−	++
8	++	+	−	−	−	−	++	−	++
9	+++	+	−	−	−	−	+++	−	++
10	++	+	−	+	−	−	++	+	+
11	++	+	−	+	−	−	++	−	+
12	++	+	−	−	−	−	++	+	+

−,+,++,+++ = estimated quantity of immunoglobulins and complement components.

30 min at room temperature. The four anti-human IgG subclass MoAb at the concentrations used in this study showed equivalent staining intensity when plasma cells in human bone marrows were examined by indirect immunofluorescence in the same manner.

RESULTS

IgG subclass deposits in MN

Table 1 records the results of immunofluorescence analysis using antibodies specific for IgG, IgM, IgA, IgG subclasses and complement components in renal biopsies from 12 patients with MN.

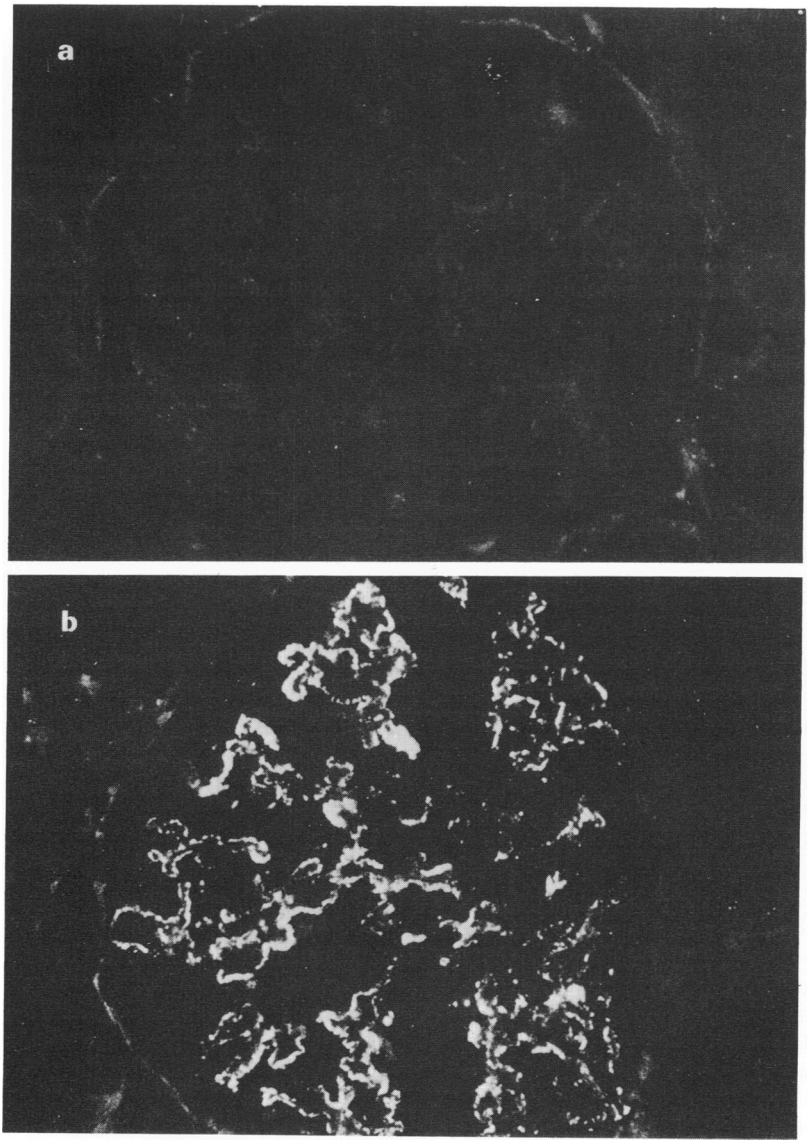

Fig. 1. Immunofluorescence microscopy of IgG subclasses in case 12. The findings of (a) (staining for IgG1) are negative. Similarly, staining for IgG2 and IgG3 are negative. Diffuse granular deposits of IgG4 are seen along the capillary loops (b). Magnification × 750.

Large amounts of fine granular deposits of IgG4 were identified along the capillary loops in all patients; seven patients also had small amounts of IgG1 deposits. There were no detectable IgG2 or IgG3 deposits in any of the patients. IgG4 was the predominant glomerular deposits in MN, and the staining patterns of glomerular deposits by anti-IgG4 antibody were quite similar to those observed with anti-IgG antibody. Clq deposits were found in four of 12 and C3 in eight of 12 patients. There was no significant correlation between the IgG subclass and complement deposits, but all patients with detectable Clq deposits also had IgM deposits.

Fig. 1 shows the immunofluorescent staining in case 12. Diffuse granular deposits of IgG4 can be seen along the capillary loops (Fig. 1b), but no IgG1 deposits are detectable (Fig. 1a). Similarly, IgG2 and IgG3 deposits were negative.

On the contrary, five renal samples without significant glomerular deposits were negative by immunofluorescence for the IgG subclasses, Clq, and C3c (data not shown).

IgG subclass deposits in other forms of GN

Patients with MPGN had coarse granular deposits of all four IgG subclasses; these were distributed both in the mesangium and along the capillary loops. In these cases, IgG1 and IgG3 were predominant. In lupus nephritis, IgG1 deposits were prevalent in all patients, IgG2 deposits were seen in biopsies from two of eight patients, IgG3 in six of eight and IgG4 in four of eight.

DISCUSSION

MN is a well characterized glomerular disease typically showing subepithelial IgG deposits along the glomerular basement membrane. The aetiology and pathogenesis of this disorder are not clear. At present there is no conclusive evidence of either *in situ* immune complex formation or circulating immune complex deposition as the basis for the pathogenesis of human MN (Cameron, 1979; Couser & Salant, 1980). In pursuit of the aetiology of MN, it is important to determine the IgG subclasses of glomerular deposition. There are controversial reports about IgG subclasses. Lewis *et al.* (1970) have reported a lack of IgG4 deposits in MN, whereas Bannister *et al.* (1983) have reported deposits of all four IgG subclasses, deposits with large amounts of IgG4, in MN. Roberts *et al.* (1983) have recently reported a predominance of IgG4 and little or no detectable IgG of the other subclasses in MN lesions in eight SLE patients, in contrast with IgG3 and IgG1 predominance in deposits present in proliferative glomerulonephritis of eight other SLE patients. In this study, we identified predominantly IgG4 glomerular deposits in all 12 patients with MN, smaller amounts of IgG1 deposits in seven patients, and few, if any, IgG2 and IgG3 deposits in all patients by using mouse MoAb to human IgG subclasses. There was a marked difference in the predominant IgG subclasses between the IgG deposits noted in MN and in MPGN and SLE. In MPGN and SLE, all four IgG subclasses were present with a predominance of IgG1 and IgG3. The predominance of IgG4 noted in the MN in our study would not appear to be artifactual because (1) IgG4 deposits were not seen in the control renal samples and in some of the SLE tissues, and IgG1 and IgG3 were predominant in MPGN and SLE nephritis; (2) at the concentrations used in this study all of the MoAb showed the same staining intensity when plasma cells in normal bone marrow were stained in the same manner and (3) the staining pattern and intensity of IgG4 were quite similar to those of IgG in MN. The differences between our results and those of others (Lewis *et al.*, 1970; Bannister *et al.*, 1983) could be due to differences between conventional antisera and MoAb, or possibly in the patient populations examined. The former possibility would appear to be the most likely, especially in view of the close parallel in our results and those reported recently by Roberts *et al.* (1983).

The four IgG subclasses differ in their concentrations in normal serum. IgG1 comprises approximately 66%, IgG2 23%, IgG3 7% and IgG4 4% of the normal total serum IgG (Yount *et al.*, 1970). It is remarkable that IgG4, which is present in only small amounts in the serum, is predominant in the glomerular deposits of MN. One possible explanation of the IgG4 predominance in GN might be that although all of the four IgG subclasses are deposited at the beginning of the disease, only IgG4 remains at the time of examination. However, it was reported that there is little difference between IgG4 and the other IgG subclasses in either the half time of

catabolism or binding activity to Fc receptors on mononuclear cells and polymorphs (Spiegelberg, 1974). This would suggest that there may be no significant difference between the clearance times for IgG4 and the other subclasses. IgG antibodies to most protein antigens are formed in quantities roughly similar to the normal IgG subclass distribution; however, carbohydrate antigens appear to elicit an immune response restricted to certain subclasses (Spiegelberg, 1974). Antibodies to coagulation factor 8 are primarily IgG4 (Andersen & Terry, 1968; Natvig & Kunkel, 1968). Therefore, another possible explanation of IgG4 predominance in MN might be that antigens in MN are restricted to certain antigens which are different from those in MPGN or SLE. Further studies are needed to address this issue.

The different IgG subclasses show different pHs in isoelectric focusing. As IgG4 is found only below pH 6·0, this subclass is anionic (Howard & Virella, 1969). There is a functionally important electrostatic interaction between circulating, charged macromolecules and the anionic components of the glomerular capillary wall (Brenner, Hostetter & Humes, 1978). Batsford, Takamiya & Vogt (1980) showed that the cationic antigen trapped in the glomerular basement membrane can lead to the *in situ* formation of immune deposits. Gauthier, Mannik & Striker (1982) reported that the positive charges on antibodies in immune complexes contributed to the deposition and persistence of the complexes. However, our data suggest that immunoglobulins in the subepithelial IgG deposits in MN are mainly anionic antibodies. According to these findings, the charges of the antigens or the loss of negative charges in the glomerular basement membrane may be important factors in MN.

Immune complexes of the IgG or the IgM class normally initiate the activation of the complement system; among subclasses of human IgG, IgG1, IgG2 and IgG3 activate Cl, whereas IgG4 does not (Ishizaka *et al.*, 1967). However, IgG4 can activate the alternative pathway of the complement system (Götze & Müller-Eberhard, 1971; Spiegelberg & Götze, 1972; Frank *et al.*, 1976). As Clq deposits were associated with IgM deposits in MN in this study, IgM antibody seems to activate the classical pathway of the complement system. We found that some MN cases with only IgG4 deposits, unassociated with the other subclasses of IgG, had C3 deposits. These findings and the similar ones of Roberts *et al.* (1983) suggest that IgG4 antibody can activate the alternative pathway of the complement system in glomerular lesions. Further studies are necessary to clarify the role of the complement system in MN.

The patients with MPGN had granular glomerular deposits of all four IgG subclasses, with a predominance of IgG1 and IgG3. Fontaine *et al.* (1980) reported that C3 nephritic factor was IgG3, a finding that may coincide with ours.

These data suggest that the IgG4 subclass may play an important role in the pathogenesis of MN. Further studies will be required to characterize the nature of IgG4 deposits in MN.

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