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Induction of Different Types of Glomerulonephritis by Monoclonal Antibodies Derived from an MRL/lpr Lupus Mouse

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MRL/Mp-lpr/lpr(MRL/lpr) lupus mice develop glomerulonephritis in which the histopathological manifestations of the disease are characterized by diffuse cell-proliferative, crescentic, and/or wire loop-like lesions, resembling those of human lupus nephritis. Although these lesions are thought to be mediated by antibodies, little data is available to explain these regular variations in glomerular lesions induced by antibodies at the monoclonal level. We studied glomerular lesions of normal or severe combined immunodeficient mice injected with nephritogenic immunoglobulin G3-producing hybridoma clones (2B11.3 and 7B6.8), which we previously established from an unmanipulated MRL/lpr mouse. Both clones caused increased serum levels of immunoglobulin G3 with identical patterns over time and both induced glomerular deposits of immunoglobulin G3 and C3. However, 2B11.3 and 7B6.8 induced glomerular lesions that differed in their histopathological manifestations. The 2B11.3 clone generated cell-proliferative lesions associated with marked Mac-2-positive macrophage infiltrates, but the 7B6.8 clone induced lesions characterized by subendothelial hyaline deposits resembling wire loops. The latter was not associated with significant inflammatory cell infiltrates at any point throughout the progression of the lesion. Thus, our findings suggest that the histopathological variation in glomerulonephritis seen in MRL/lpr mice results from clonally expanded B cell clones that produce nephritogenic antibodies with different pathogenic potencies. (Am J Pathol 1993, 143:1436–1443)

MRL/Mp-*lpr/lpr* (MRL/lpr) mice spontaneously develop a lethal glomerulonephritis (GN) in association with autoimmune traits.[1,2] The histopathological manifestations of glomerular lesions in these mice are diffuse cell-proliferative, crescentic, and/or wire loop-like in type, resembling those seen in human lupus nephritis. It has been thought that the glomerular lesions in these mice are mediated by circulating immune complexes associated with autoantibodies such as anti-DNA antibodies, rheumatoid factors, and/or anti-glycoprotein 70 antibodies[3–5] but few studies of the antibodies responsible have been carried out at the monoclonal level.[6]

In previous studies, we found that immunoglobulin G3 (IgG3) production plays a critical role in the development of GN in MRL/lpr mice[7] based on the following observations: 1) IgG3 is the major subclass of IgG in kidney-extracts from MRL/lpr mice despite the predominance of IgG2a in serum, and 2) the messenger RNA level of IgG3 in spleen cells correlates well with the severity of GN among the hybrid mice, MRL/lpr × (MRL/lpr × C3H/lpr)F1, in which the genes for GN must be segregated.[8] Furthermore, we recently succeeded in establishing hybridomas producing IgG3 antibodies from an unmanipulated MRL/lpr mouse that were capable of inducing glomerular lesions when injected into normal mice.[9]

In this study, we analyzed the histopathological and immunohistochemical manifestations of glomerular lesions induced by these nephritogenic IgG3-producing hybridoma clones in normal or severe combined immunodeficient (SCID) mice. We found that the antibodies themselves induce glomerular lesions and do so with different histopathological manifestations. Our results indicate that GN of MRL/lpr mice may be caused by expansion of B cell clones producing specific antibodies with different pathoge-

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netic potencies, which may lead to regular variations in the type of glomerular lesion developed.

Materials and Methods

Mice

MRL/lpr and BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and from Japan Clea, Ltd. (Tokyo, Japan), respectively, and were bred and housed in the clean rooms of the Animal Research Institute of Tohoku University School of Medicine. From these, we developed a strain of hybrid mice, (MRL/lpr × BALB/c)F1. C.B-17/Icr *scid/scid* (SCID) mice[10] were kindly donated by Dr. S. Ikehara (Kansai Medical University, Japan).

Animal Injections of Hybridomas Producing IgG3 Antibodies

Three IgG3-producing hybridoma clones, 2B11.3, 7B6.8, and 1G3, were used in these experiments. These had been established in our recent study.[9] In brief, NS-1 myeloma cells derived from BALB/c mice were used for cell fusion with spleen cells from an unmanipulated 20-week-old male MRL/lpr mouse. IgG3-producing hybridoma cell lines were identified by enzyme-linked immunosorbent assay using HRP-labeled rabbit anti-mouse IgG3 antibodies. The 2B11.3 and 7B6.8 clone induced glomerular injury when injected into (MRL/lpr × BALB/c)F1 normal mice, but the 1G3 clone did not.[9] In the present experiments, the 1G3 clone was used for a negative control.

Each hybridoma clone (1×10^7 cells, each of 2B11.3, 7B6.8, or 1G3) was injected intraperitoneally into 12-week-old (MRL/lpr × BALB/c)F1 mice that had been administered 2, 6, 10, 14-tetramethyl-pentadecane (Aldrich Chem., Inc., Milwaukee, WI). In histological studies, we had confirmed that (MRL/lpr × BALB/c)F1 mice did not spontaneously develop glomerular lesions before 24 weeks of age. In some experiments, these clones were injected into 8- to 12-week-old SCID mice under the same conditions. On days 10 to 25 after the injection, serum samples were collected from these mice under ether anesthesia, and kidneys were removed for examination. In the case of SCID mice, heart, lungs, liver, pancreas, and salivary glands were removed as well.

Amounts of IgG3 in Serum

To quantify IgG3 in serum of the mice injected with the IgG3 antibody-producing hybridomas, single radial immunodiffusion was performed according to a method described elsewhere.[11] One percent agarose in 0.1 mol/L phosphate-buffered saline, pH 7.2, containing rabbit anti-IgG3 serum (ICN ImmunoBiologicals, Lisle, IL) was prepared and spread on a glass plate. Samples of 4 μl each were subjected to 1.5-mm diameter punched holes and incubated at room temperature for 48 hours. Precipitin rings stained with amido black 10B were measured. The amounts of IgG3 contained in the serum samples were estimated from a standard curve using mouse IgG subclass standards (Miles Laboratories Inc., Naperville, IL). The anti-mouse IgG3 sera used here had been confirmed in our previous study[7] to be specific for the IgG3 subclass by single radial immunodiffusion.

Histopathological Examinations

Kidney samples obtained from three to five mice per group were fixed with 10% formalin in 0.01 mol/L phosphate buffer (pH 7.2) and embedded in paraffin. They were stained with hematoxylin and eosin, periodic acid-methenamine-silver, phosphotungstic acid hematoxylin, or periodic acid-Schiff for histological examination by light microscopy. For electron microscopy, small pieces of renal cortices were fixed in 2.5% glutaraldehyde overnight at 4 C and postfixed in 2% osmium tetroxide for 2 hours at 4 C. After dehydration with ethanol, they were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead nitrate and then examined under an electron microscope, Nihon Denshi, JEM-1200EX.

Immunohistochemical Examinations

Procedures for immunohistochemical study were based on a method described elsewhere.[12] In brief, tissue samples obtained at autopsy were frozen in OCT compound (Miles Inc., Elkhart, IN). Cryostat tissue sections were cut to a thickness of 3 μ and fixed in cold acetone for 5 minutes. C3 was detected by a direct method using fluorescein isothiocyanate-conjugated rabbit anti-mouse C3 (Miles Laboratories). The staining of IgG3 was performed with rabbit anti-mouse IgG3 serum (ICN ImmunoBiologicals, Lisle, IL) and followed with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Miles).

To detect Mac-2 antigens,[13] kidney samples were fixed with periodate-lysine-paraformaldehyde solution[14] overnight at 4 C before freezing. Immunostaining was performed using rat anti-Mac-2 antibodies (Hybritech Inc., San Diego, CA), biotinylated rabbit anti-rat IgG antibodies and HRP-labeled avidin-biotin complex (ABC kit PK400, Vector Laboratories Inc., Burlingame, CA) or fluorescein isothiocyanate-labeled avidin (Vector). For quantitative analysis, Mac-2-positive cells were counted in a total of 20 glomeruli (± SD) from each mouse under light microscopy, and the data was represented as a Mac-2-positive cell index.

Results

Increase of the Serum IgG3 Levels in the Hybridoma-Injected Mice

We first examined the serum IgG3 levels in (MRL/lpr × BALB/c) F1 mice injected with the three hybridoma clones, 2B11.3 and 7B6.8 (nephritogenic) and 1G3 (non-nephritogenic). The serum level of IgG3 had risen to more than 1.0 mg/ml on day 15 after injection in all groups. As shown in Figure 1, changes over time of IgG3 levels in serum obtained from the injected mice were almost identical for both 2B11.3 and 7B6.8 clones.

Analysis of Glomerular Lesions Generated by 2B11.3 and 7B6.8 Clones

Animals injected with 2B11.3, 7B6.8, or 1G3 clone developed ascites and died on days 16 to 18, 23 to 25, or 20 to 25 after the injection, respectively. The representative histopathological manifestations on

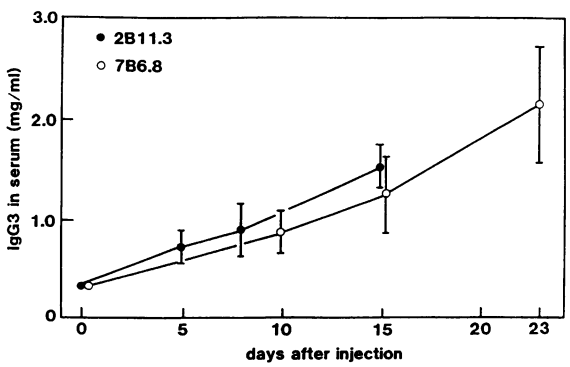

Figure 1. *Time course of the changes in IgG3 level in serum from (MRL/lpr × BALB/c) F1 mice injected with the 2B11.3 or 7B6.8 clone. Each point represents the mean of five to nine mice. Each bar indicates ± SD.*

day 15 or 20 of glomerular lesions induced by clone 2B11.3 or clone 7B6.8, respectively, are shown in Figure 2, a and b. In the case of clone 1G3, the injected mice did not develop any glomerular lesions before death (Figure 2c).

The glomerular changes induced by the two nephritogenic clones were significantly different. Clone 2B11.3 induced diffuse cell-proliferative type lesions, associated with inflammatory cell infiltrates and consequent lobular changes in glomeruli (Figure 2a). Clone 7B6.8, on the other hand, induced wire loop-like lesions, characterized by severe hyaline material deposits along the glomerular capillary walls and sometimes by hyaline thrombi (Figure 2b). These two types of lesions somewhat resembled the glomerular lesions in MRL/lpr mice, as shown in Figure 2d.

Neither type of glomerular lesion induced significant thickening of the glomerular basement membrane as observed by periodic acid-methenamine-silver staining (Figure 3, a and c). The glomerular lesions caused by clone 2B11.3 were characterized by an accumulation of Mac-2-positive cells, as seen in immunohistological studies (Figure 3b), suggesting that activated macrophages are involved in the cell-proliferative type of lesion. On the other hand, very few of the infiltrating cells present in lesions induced by clone 7B6.8 were Mac-2-positive. The hyaline deposits induced by 7B6.8 were positively stained with phosphotungstic acid hematoxylin (Figure 3d), suggesting that they contained fibrin components.

Electron microscopic findings were consistent with the results detailed above. In the glomerular lesions induced by the 2B11.3 clone, infiltrated cells were composed of neutrophils and macrophages, some of which contained electron-dense granules within phagocytic granules (Figure 4a). Granular electron-dense deposits were sometimes observed in subendothelial and mesangial regions and in endothelial cells (not shown). On the other hand, in the case of clone 7B6.8, there was no accumulation of macrophages or neutrophils. Electron-dense deposits were localized mainly in subendothelial and mesangial regions and sometimes occupied the capillary lumen (Figure 4b).

IgG and C3 Deposits in Glomerular Lesions Induced in SCID Mice

In immunohistological studies, IgG3 and C3 deposits were observed on day 15 or on day 20 in glomeruli of the hybrid mice injected with either the 2B11.3

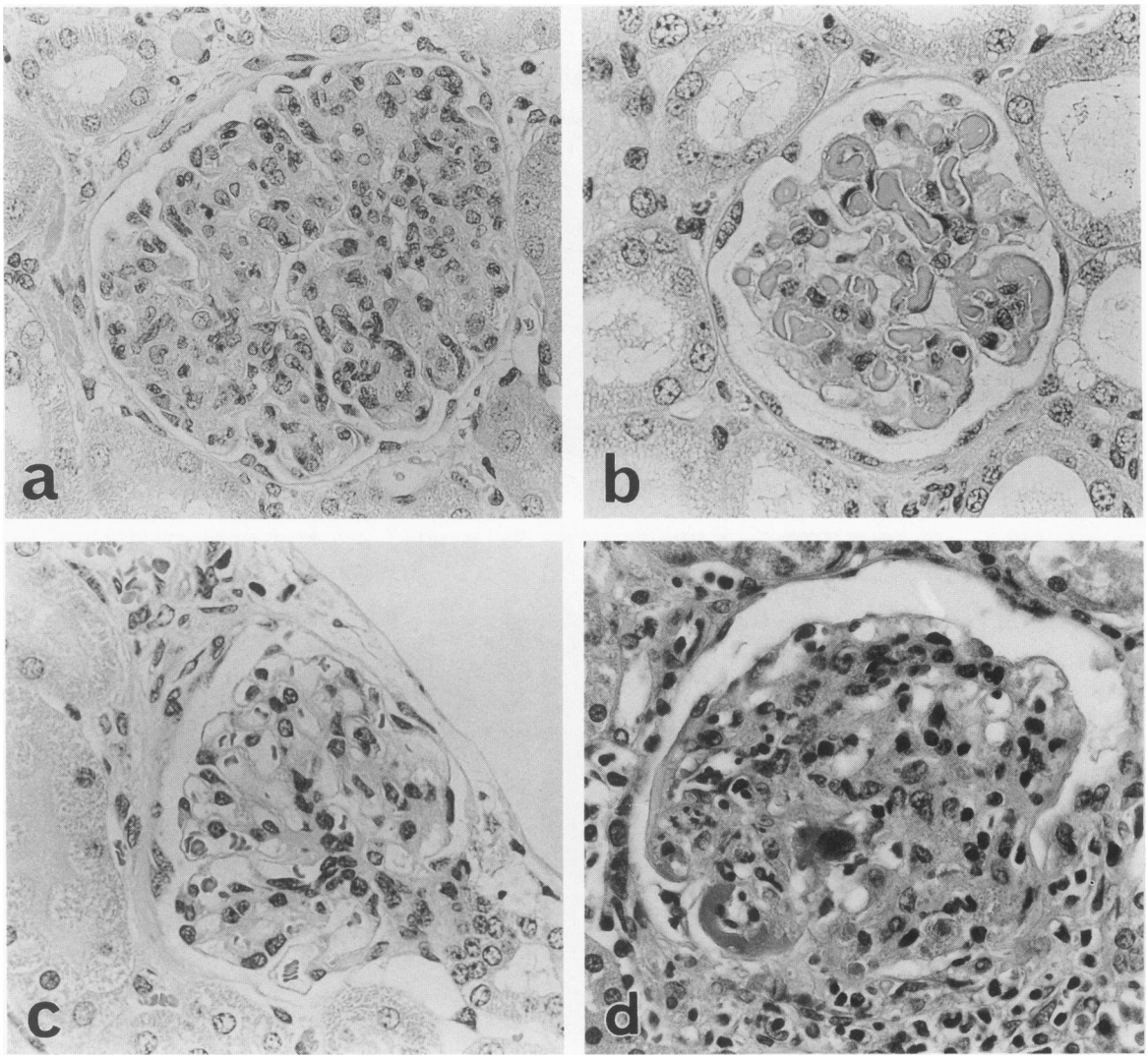

Figure 2. a: *Representative histopathological manifestations on day 15 of glomerular lesions of (MRL/lpr × BALB/c) F1 mice induced by the 2B11.3 clone, characterized by extreme cell proliferation with an accumulation of a number of macrophage-like and polymorphonuclear cells. This type of lesion is termed cell-proliferative. (H&E, 400×).* b: *Typical histopathological features of glomerular lesions on day 20 of (MRL/lpr × BALB/c) F1 mice induced by the 7B6.8 clone, manifesting hyaline deposits along the capillary wall and partially hyaline thrombi in the capillary lumen. This type of lesion is designated wire loop-like. (H&E, 400×).* c: *Clone 1G3 had not generated any significant lesions in glomeruli of (MRL/lpr × BALB/c)F1 mice by on day 20. (H&E, 400×).* d: *GN in a 27-week-old MRL/lpr male mouse. Presented here are typical histopathological features of GN in this strain of mice, characterized by cell-proliferative and wire loop-like lesions (lower left). (H&E, 400×).*

or 7B6.8 clone, respectively (data not shown). To clarify whether these IgG3 deposits had originated from the hybridomas or represented IgG3 synthesized by the recipient mice, we induced glomerular lesions in SCID mice by injecting them with the hybridomas. Because SCID mice are severely deficient in immunoglobulin stem cells and are hypogammaglobulinemic,[10] any of the deposited IgG3 would have to be from the implanted hybridomas. As shown in Figure 5, IgG3 deposits were clearly present in glomeruli of SCID mice injected with both hybridomas and were associated with C3 deposition. In the case of clone 2B11.3, a pattern of micronodular deposits of IgG3 or C3 was the dominant feature, whereas linear deposits along the capillary wall were the main finding in glomerular lesions induced by clone 7B6.8. These patterns may correspond to the distribution of electron-dense deposits observed by electron microscopy.

It was interesting to note that the histopathological lesions observed in the SCID mice by light microscopy were almost identical to those in the hybrid mice: a cell-proliferative type in clone 2B11.3 and a wire loop-like type in clone 7B6.8 (data not

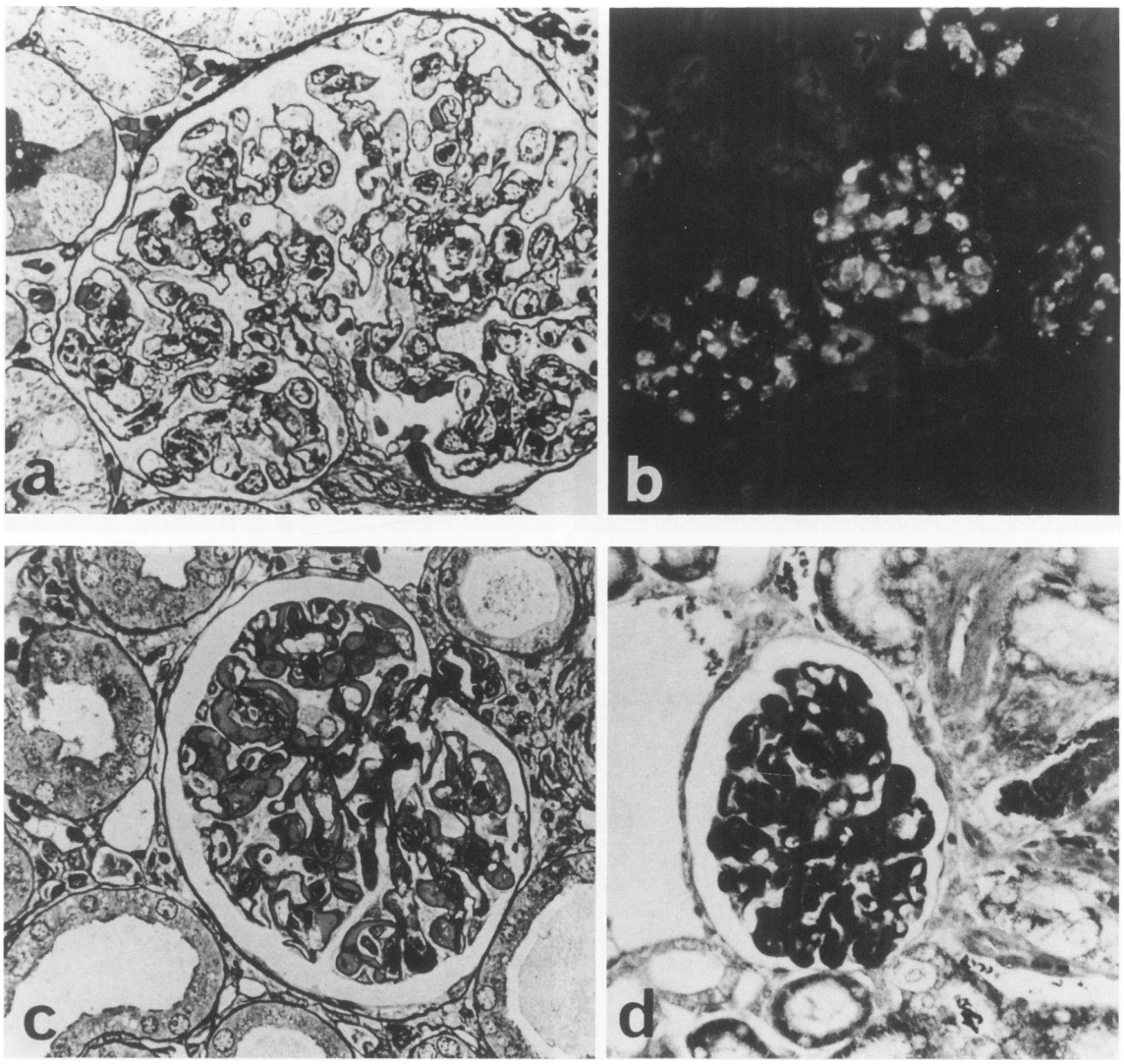

Figure 3. *Microscopic findings of glomerular lesions of (MRL/lpr × BALB/c) F1 mice injected with the 2B11.3 or 7B6.8 clone.* a *and* c: *Neither glomerular lesion is associated with thickening of the glomerular basement membrane.* a: *2B11.3 on day 15;* c: *7B6.8 on day 20. (periodic acid-methenamine-silver, 700×).* b: *A number of Mac-2-positive cells, indicating activated macrophages, have accumulated in a glomerulus in the case of clone 2B11.3 (day 15) (immunofluorescence, 300×).* d: *Phosphotungstic acid hematoxylin staining reveals a fibrin component deposit in glomerular lesions induced by clone 7B6.8 (day 20) (400×).*

shown). Moreover, no significant deposition of IgG3 or C3 was observed in any other organs of SCID mice injected with both clones. This indicates that IgG3 originating from the hybridomas was selectively deposited in glomeruli.

Temporal Development of Glomerular Lesions after the Hybridoma Injection

To study the temporal evolution of glomerular lesions after the hybridoma injection, kidneys of the hybrid mice were evaluated at varying intervals after injection. In mice injected with 2B11.3 cells (n = 3 to 5) and studied 5, 8, 11, and 15 days later, minimal changes, characterized by slight mesangial expansion and inflammatory cell infiltration in glomeruli, were detected with a light microscope 8 days after hybridoma injection, and this was followed by the development of cell-proliferative glomerular lesions in all mice before day 15.

In the case of mice injected with 7B6.8 (n = 3 to 5), kidneys were examined on day 10, 15, 20, or 23 because the development of ascites was slower. The initial changes of glomeruli were barely detected on day 15 with a light microscope, and these consisted only of the appearance of small quantities of acidophilic and amorphous deposits in the capillary wall. Significant changes in renal glomeruli characterized by wire loop-like lesions were found on day 20. Throughout the progression of these

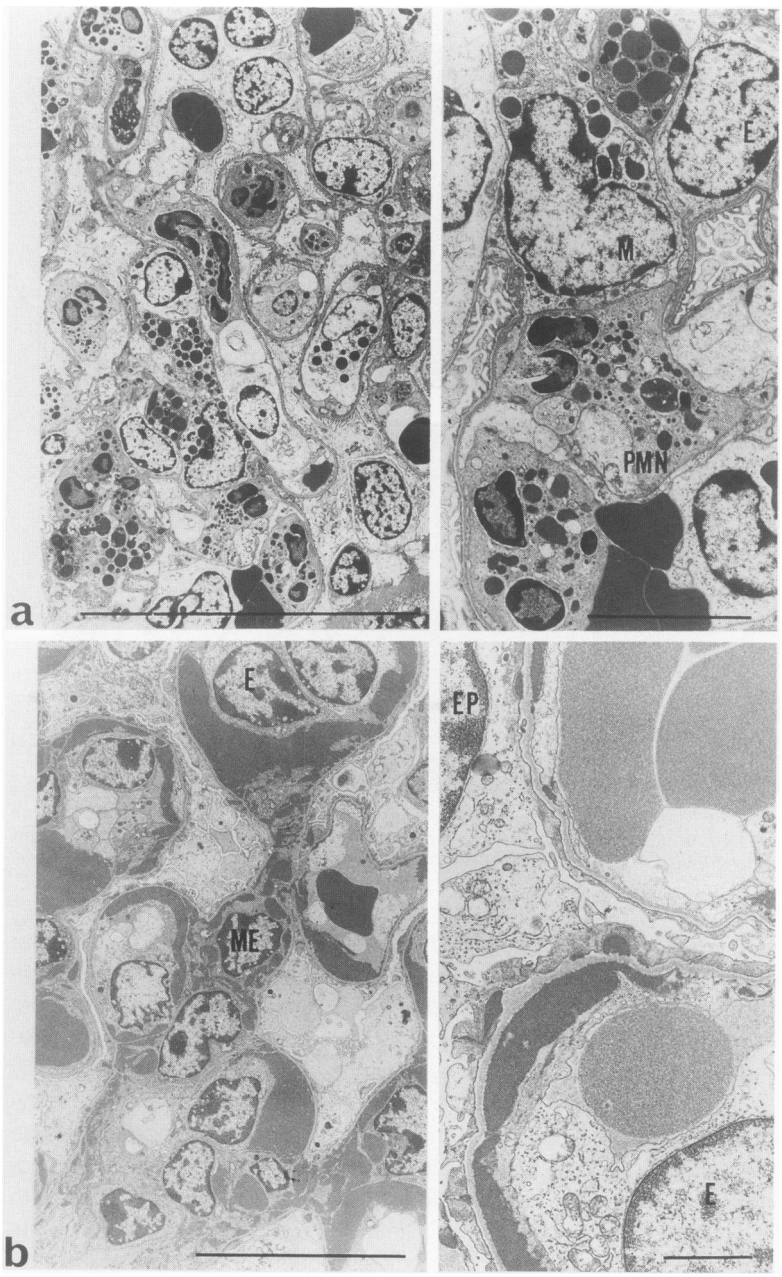

Figure 4. *Representative electron micrographs of the glomerular lesions of the injected (MRL/lpr × BALB/c) F1 mice.* **a:** *Clone 2B11.3 on day 17: many polymorphonuclear cells and macrophages phagocytizing osmiophilic material can be observed in a glomerulus.* **b:** *Clone 7B6.8 on day 22: osmiophilic deposits are marked in subendothelial and mesangial regions, resulting in partial obstruction of the capillary lumen. Similar deposits are occasionally localized in subepithelial regions. No inflammatory cell infiltration can be observed. PMN: polymorphonuclear cell, M: macrophage, E: endothelial cell, ME: mesangial cell, EP: epithelial cell. Scale bar indicates 2 μ.*

glomerular lesions, none of the cell-proliferative type was observed in this group.

Differences in glomerular lesions between mice injected with these clones became more obvious when quantitative studies of Mac-2-positive cells present in the glomeruli were performed (see Materials and Methods). Three to five mice from each group were examined on each day and the Mac-2 index determined. Mac-2-positive cells, which indicate the presence of activated macrophages,[13] had markedly accumulated in the glomerular lesions induced by clone 2B11.3 (Mac-2-positive cell index on day 15, 11.8 ± 1.3), but were not present in significant amounts in lesions induced by clone 7B6.8 at any point throughout the progression (Mac-2-positive cell index on day 23, 1.7 ± 1.9).

Discussion

In the present study, we found that two hybridoma clones obtained from an unmanipulated MRL/lpr mouse could generate different types of glomerular lesions when injected into normal or SCID mice.

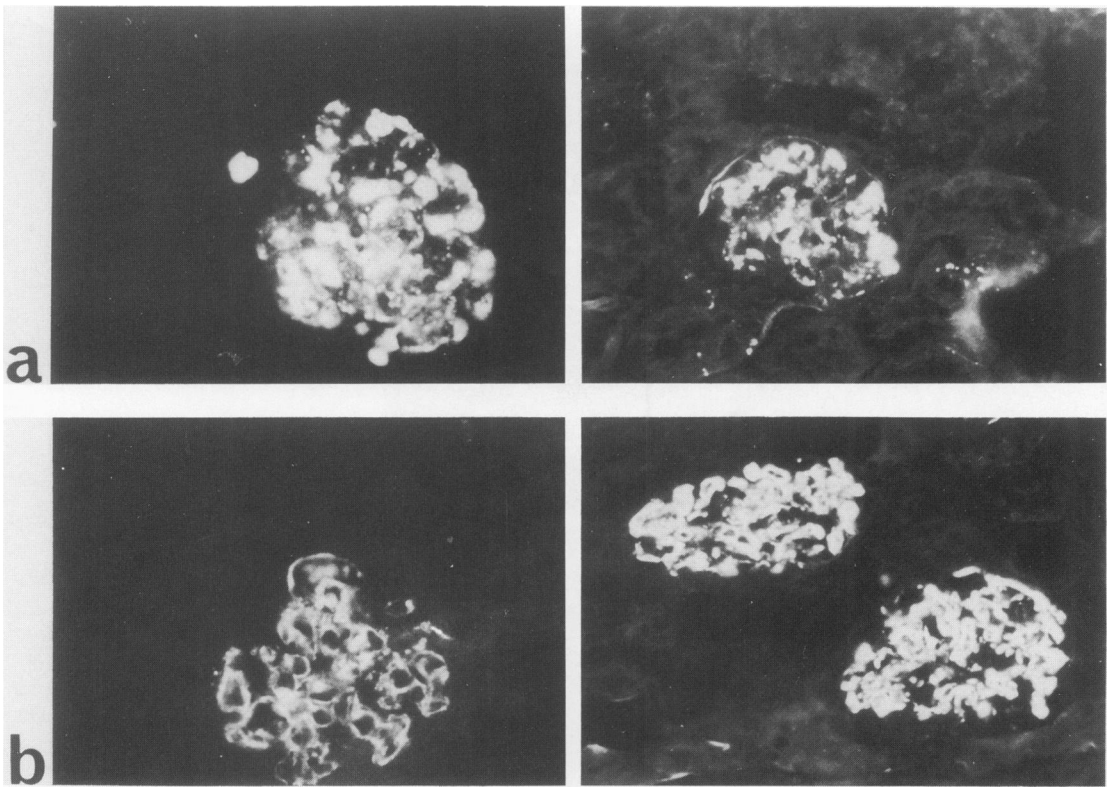

Figure 5. *Affected glomeruli in the injected SCID mice reveal IgG3 (left) and C3 (right) deposits (immunofluorescence, 300×).* a: *Clone 2B11.3 on day 15: IgG3 deposits in a micronodular form are observed, suggesting phagocytized IgG3. Granular deposits of C3 are also significant.* b: *Clone 7B6.8 on day 20: IgG3 deposits along the capillary wall, partially involving the capillary lumen. The pattern of C3 deposition is identical to that of IgG3.*

The histopathology induced by the 2B11.3 hybridoma clone was cell-proliferative, whereas that of the 7B6.8 clone was wire loop-like. Both histopathological features are characteristic for GN of MRL/lpr mice and are also seen in human lupus nephritis.

The glomerular lesions induced by clone 2B11.3 were associated with IgG3 deposits, and to a lesser extent C3 deposits, and were accompanied by macrophage and polymorphonuclear cell infiltrates. On the other hand, 7B6.8 injection caused severe hyaline deposits in glomerular subendothelial regions, and inflammatory cell infiltrates in glomeruli were not significant at any point throughout the progression of the lesions. These findings strongly suggest that the mechanisms for induction of glomerular injury are quite different for each of these two clones. Furthermore, the additional IgG3 hybridoma clone, 1G3, did not produce any definable pathology, indicating that the pathology observed with the two nephritogenic clones was not simply due to the presence of IgG3-producing hybridomas in the mice.

IgG3 produced from both clones, 2B11.3 and 7B6.8, seemed to selectively deposit in glomeruli, but not in other organs, and resulted in glomerular injury. It was clear that the deposited IgG3 originated from the injected hybridomas and not from the host, because the same pattern of injury was reproduced by injecting SCID mice that congenitally lack immune responses and have extremely low levels of serum immunoglobulin.[10] Moreover, the use of SCID mice made it clear that the IgG3 monoclonal antibodies did not require any interaction with host-derived immunoglobulins, including anti-idiotype antibodies, to generate glomerular lesions.

The fact that the types of lesion seen with the two hybridomas are different might simply be related to the antigen specificity of the monoclonal antibodies. Our previous investigations show that 7B6.8 antibodies, but not those of 2B11.3, have ss- and ds-DNA binding activities, and that both antibodies lack rheumatoid factor activity.[9] An IgG3 rheumatoid factor-producing hybridoma clone derived from an MRL/lpr mouse, 6–19, also generates glomerular lesions, the histopathological features of which resemble those described for clone 7B6.8.[6] Furthermore, Reininger et al[15] replaced the native light chains of 6–19 with chains derived from λ 1 derived from the J558L myeloma and found that these chi-

meric 6–19 antibodies could still induce glomerular lesions although they no longer had rheumatoid factor activity. Thus, in the case of the 6–19 antibodies, it seems that antigen specificity was not a critical determinant of glomerular pathogenicity. This further suggests that the capacity to form either circulating or *in situ* immune complexes via standard immune complex formation may not be essential for inducing glomerular injury. The nephritogenic IgG3 antibodies, including 2B11.3 and 7B6.8, may deposit in glomeruli via different mechanisms, but at least not via ones that are immune complex-mediated.

Two types of histopathological features of glomerular lesions generated by these antibodies were observed simultaneously in glomeruli of MRL/lpr mice. This indicates that glomerular lesions in these mice likely result from a combination of expanded B cell clones producing antibodies with different pathogenetic potencies. We can further speculate that when a nephritogenic antibody-producing clone such as 7B6.8 expands during the progress of glomerulonephritis, the lesions change to or are modified by the wire loop type. Thus, the regular histopathological variations in glomerular lesions in MRL/lpr mice may be determined by which nephritogenic antibody-producing B cell clones expand. This idea may also be applicable to investigations into human lupus nephritis.

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