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A Transgenic Mouse Model of Autoimmune Glomerulonephritis and Necrotizing Arteritis Associated with Cryoglobulinemia

Shuichi Kikuchi,2* Yves Pastore,2* Liliane Fossati-Jimack,2* Aki Kuroki,* Haruyoshi Yoshida,† Thierry Fulpius,* Kimi Araki,* Satoru Takahashi,* Robert Lemoine,† Luc Reininger,‡ and Shozo Izui3*

Mice implanted with hybridoma secreting 6-19 IgG3 anti-IgG2a rheumatoid factor (RF) with cryoglobulin activity develop acute glomerulonephritis and cutaneous leukocytoclastic vasculitis. As the RF activity is implicated in the skin, but not glomerular lesions, it is still unclear whether the renal pathogenicity is determined by 6-19 H chains alone or their combination with L chains. To address this question, we have generated transgenic mice expressing only the H chain gene expressing only the H chain gene or both H and L chain genes of the 6-19 IgG3 anti-IgG2a RF and determined the development of glomerular and vascular lesions. H-single and H/L-double transgenic mice displayed comparable high amounts of IgG3 cryoglobulins, but only H/L-double transgenic mice having 10-fold higher levels of IgG3 anti-IgG2a RF progressively developed chronic, lethal glomerulonephritis. The severe glomerular lesions observed at 8–10 mo of age were very heterogeneous (membranoproliferative changes, crescents, and sclerosis); in addition, one-third of them had necrotizing arteritis in the kidneys and skeletal muscles. These renal and vascular changes were very different from those observed in the acute cryoglobulinemia, characterized by mainly “wire-loop” glomerular lesions and a cutaneous leukocytoclastic form of vasculitis. Thus, our data demonstrate the importance of a unique combination of the H and L chains for the expression of the pathogenic activity of IgG3 cryoglobulins and that a single autoantibody is able to induce different types of glomerular and vascular complications, depending on its production levels and kinetics. The Journal of Immunology, 2002, 169: 4644–4650.

Cryoglobulins are Igs that undergo reversible precipitation in the cold. Pathological cryoglobulinemia occurs in lymphoproliferative disorders, autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis, or a variety of chronic infectious diseases (1, 2). Conventionally, cryoglobulins are classified as type I or type II: type I cryoglobulins are usually of chronic infectious diseases (1, 2). Conventionally, cryoglobulins are classified as type I or type II: type I cryoglobulins are usually monoclonal IgM or IgG and type II cryoglobulins are rheumatoid factors (RF)3 which form cryoprecipitating complexes with polyclonal IgG. The presence of these cryoglobulins could result in a wide range of vascular, renal, and neurological complications. However, the precise cellular and molecular mechanisms involved in the induction of the cryoglobulin-associated pathology have not been well defined.

The unique property of Igs of the IgG3 isotype to form self-associating complexes as a result of nontypical IgG3 Fc–Fc interaction (3, 4) is apparently responsible for the cryoglobulin activity observed with some IgG3 myeloma proteins in humans (5–7) and a majority of IgG3 mAb in mice (8–10). Significantly, the implantation of hybridoma cells secreting IgG3 anti-IgG2a RF derived from lupus-prone MRL-lpr/lpr mice rapidly induced acute glomerulonephritis characterized by “wire-loop”-like glomerular lesions and cutaneous leukocytoclastic vasculitis (11, 12). Studies with hybridoma secreting 6-19 IgG3 anti-IgG2a RF mAb have demonstrated the development of glomerular, but not cutaneous vascular lesions in Ig-deficient mice lacking the corresponding IgG2a autoantigens (13, 14), while its IgM and IgG1 switch variants lacking cryoglobulin activity failed to induce both tissue lesions (10, 15). Thus, both RF and cryoglobulin activities, i.e., cryoglobulin IgG3-IgG2a immune complexes, are required for the development of skin vasculitis, while renal pathogenicity is not dependent on the anti-IgG2a RF property. This conclusion was further supported by the finding that glomerular, but not cutaneous, lesions were similarly induced by a hybrid IgG3 mAb made of the 6-19 IgG3 H chains and the L chains from J558 anti-γδ-3 dextran Abs devoid of the RF activity (13). However, it is still unclear whether the renal pathogenicity of the 6-19 RF mAb is determined by a particular physicochemical property of the H chain responsible for cryoglobulin activity or by a combined action of the H and L chains potentially conferring an additional ligand-binding property other than RF activity.

We have generated transgenic mice expressing the H chain alone or both H and L chains of the pathogenic 6-19 IgG3 RF mAb to address this question. In the H chain single-transgenic mice, the L chains of the transgenic IgG3 Abs are heterogeneous, whereas in the double-transgenic mice the majority of the IgG3 Abs carry identical L chains derived from the transgene. These mice should allow us to determine whether the presence of particular IgG3 cryoglobulins consisting of 6-19 H chains and a large variety of L chains is a nephritogenic condition, or whether the combination of the 6-19 H and L chains is critical for the renal pathogenicity. Furthermore, immunopathological consequences associated with the chronic presence of the pathogenic 6-19 cryoglobulins can be compared with those acutely induced as a result of their rapid elevation of very high levels in sera following the implantation of 6-19 hybridoma cells. We report herein the development of chronic glomerulonephritis with highly heterogeneous lesions only in the 6-19 H and L chain double-transgenic (6-19-H/L) mice,

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suggesting the importance of a unique combination of the 6-19 H and L chains for the renal pathogenicity. In addition, the 6-19-H/L mice developed necrotizing arteritis in kidneys and skeletal muscles, rather than the cutaneous vasculitis which occurs following the implantation of 6-19 hybridoma cells. These results demonstrate remarkable differences in the pathogenic potential of a single autoantibody, depending on its production levels and kinetics.

Materials and Methods

**DNA constructions**

The LVDJH6-19-Cy3 and LVJ6-19-Ck plasmids containing the complete 6-19 IgG3 H or L chain genes, respectively, were constructed using the following DNA fragments: the rearranged LVDJH or LVJ region isolated from CDNA encoding the H or L chain of the 6-19 mAb (13), the promoter region isolated from pSV-Vµ1 (16), the H chain enhancer isolated from pSV2-neo (a kind gift from Dr. K. Rajewsky, Köln, Germany), the Cy3 region derived from the genomic clone pFW7 (17) and the Ck region derived from pEHV-Ck-neo (a kind gift from Dr. K. Rajewsky).

**Generation of 6-19 IgG3 H chain and L chain transgenic mice**

The LVDJH6-19-Cy3 or LVJ6-19-Ck plasmids containing the complete 6-19 IgG3 H or L chain gene was microinjected into fertilized eggs of C57BL/6 x DBA/2F1 mice. Mice were screened for the 6-19 IgG3 H chain transgene by determining serum levels of IgG3 by ELISA and was confirmed by Southern blot analysis. A founder expressing the 6-19 H chain transgene, designated 6-19-H, has been established and used in the present study. For the 6-19 L chain transgene, mice were first screened by Southern blot analysis and then mice bearing the transgene were crossed with the 6-19-H transgenic mice. Their offspring were tested for the expression of both H and L chain transgenes by measuring serum levels of IgG3 anti-IgG2a RF activities by ELISA. Based on this analysis, a 6-19 L chain transgenic founder, designated 6-19-L, was established. The 6-19-H and 6-19-L mice contain ~20 and 10 copies of the respective transgenes, which were stable inbred as determined by PCR analysis. The development of splenic B cells was assessed by staining spleen cells with anti-mouse 

**Southern blot and PCR analysis**

DNA was prepared from tails of 4-wk-old mice, digested with EcoRI, electrophoresed on a 1% agarose gel, transferred to a nylon membrane, and hybridized to the 6-19-Vµ1 or V region of κ chains probe. For PCR amplification of the transgene, the following primers were used: promoter primer (5'-CAGTTCTCTTACAGTTA-3'), JH6-19 primer (5'-CTCACCCTGTAGGAGACTGGT-3') for the 6-19 H chain transgene, and JH6-19 primer (5'-ACTACTACGTITTATTCACAGC-3') for the 6-19 L chain transgene.

**Monoclonal Ab**

Hybridoma secreting 6-19 IgG3 anti-IgG2a RF or 9A6 IgG3 anti-dNMP mAb was established from unmanipulated MRL-lpr/lpr mice (11) and from BALB/c mice immunized with DNP-LPS (9), respectively. A hybrid IgG3 mAb made of the H chains of the 6-19 mAb and the L chains from the 9A6 anti-dNPP mAb (6-19H9A6L) was generated by mixing 9A6 H chain loss mutant cells with the LVDJH6-19-Cyk plasmid along with a plasmid containing the hygromycin-resistant gene. After selection for resistance to hygromycin and secretion of IgG3 Abs, a stable transfected cell line was established.

**Serological assays**

Serum levels of IgG3 anti-IgG2a RF were determined by ELISA as described elsewhere (12). Briefly, microtiter plates were coated with 4-hydroxy-3-iodo-5-nitrophenylacetyl (NIP)-conjugated BSA and subsequently incubated with IgG2a anti-NIP (NIP-23) mAb before the addition of the test samples. The assay was developed with alkaline phosphatase-labeled rat anti-mouse γ3-chain mAb (H139.61.1) (18). Results are expressed in milligrams per milliliter in reference to a standard curve obtained with purified IgG3 6-19 RF mAb. IgG3 and IgG2a concentrations in sera and cryoglobulins were quantitated as described previously (12, 19). Serum levels of IgG3 anti-neutrophil cytoplasmic autoantibody (ANCA) was measured by IFA, in which microtiter plates coated with human myeloperoxidase (Calbiochem-Novabiochem, La Jolla, CA) were incubated with serum samples at a 1/100 dilution, followed by labeled rat anti-mouse γ3-chain mAb,

**Histopathology**

Kidneys and other major organs were obtained at autopsy, and histological sections were stained with periodic acid-Schiff (PAS) or H&E. Glomerulonephritis was scored on a 0–4 scale based on the intensity and extent of histological changes according to Pirani and Salinas-Madrail (20). A grade 0 was given to kidneys without glomerular lesions. The 1+ lesions corresponded to minimal thickening of the mesangium, 2+ lesions contained noticeable increases in both mesangial and glomerular cellularity, 3+ lesions were characterized by the preceding conditions with superimposed inflammatory exudates and capular adhesions, and in 4+ lesions the glomerular architecture was obliterated in >70% of glomeruli and tubular cast formation was extensive. Grades 3 and 4 glomerulonephritis were considered to be significant contributors to clinical disease and/or death. Glomerular and vascular deposition of IgG3 and IgG2a was determined by staining frozen kidney sections with rat anti-IgG3 (H139.61) or anti-IgG2a IgG(1a,8.3) mAb, followed by FITC-labeled goat anti-rat Ig conjugates (Vector Laboratories, Burlingame, CA). Polyclonal rat IgG purified from a pool of normal rat serum was used as a control. C5 deposits were examined by direct staining with FITC-labeled goat anti-mouse C3 conjugates (Cappel Laboratories, West Chester, PA). The nature of infiltrating mononuclear cells in small- and medium-sized arteritis was determined by staining with rat anti-IgG3, rat-anti-CD4 (GK1.5), rat anti-CD8 (H35), or rat anti-Mac-1 (M1/70) mAb, followed by FITC-labeled goat anti-rat Ig conjugates.

**Transplantation of hybridoma and transfectoma cells**

Because the 6-19 hybridoma was derived from a fusion of MRL spleen cells (H-2k) with BALB/c myeloma cells (H-2b), 105 hybridoma cells were injected i.p. into pristane-treated transgenic mice along with a mixture of anti-CD4 (GK1.5) and anti-CD8 (H35.17.2) mAb (1 mg of each mAb) to avoid their rejection. In some experiments, 6-19 hybridoma and 6-19H9A6L transfectoma cells were inoculated i.p. or s.c. into (MRL × BALB/c)F1 mice.

**Statistical analysis**

Statistical analysis was performed with the Wilcoxon two-sample test. Values of p > 0.05 were considered to be insignificant.

**Results**

Comparable development of IgG3 cryoglobulinemia, but differential IgG3 anti-IgG2a RF autoantibody production in 6-19-H single- and 6-19-H/L double-transgenic mice

6-19-H and 6-19-L mice with a mixed C57BL/6 and DBA/2 genetic background were intercrossed, and the 6-19-H, 6-19-L, 6-19-H/L, and nontransgenic littermates were identified by PCR analysis. At 2 mo of age, serum levels of IgG3 were markedly elevated in both 6-19-H and 6-19-H/L mice (Fig. 1). Their mean values were 2.2 and 2.4 mg/ml, respectively, which were more than 10 times higher than those in 6-19-L (0.2 mg/ml) and nontransgenic littermates (0.3 mg/ml). In parallel to the increased IgG3 levels, sera from both transgenic mice expressing the 6-19 H chains exhibited comparable IgG3 cryoglobulin activities (6-19-H, 28.3 μg/ml; 6-19-H/L, 29.6 μg/ml), while cryoglobulins were undetectable (<0.1 μg/ml) in sera from the 6-19-L and nontransgenic littermates (Fig. 1). These IgG3 levels remained constant, at least until 1 year of age (data not shown). It should be mentioned that serum levels of IgG2a at 2 mo of age were significantly lower in both 6-19-H/L (2.1 ± 1.0 mg/ml) and 6-19-L mice (2.3 ± 0.9 mg/ml), as compared with those in 6-19-H (3.3 ± 0.8 mg/ml) and nontransgenic mice (3.8 ± 0.9 mg/ml). This was likely to be related to a lower number of mature B cells in spleen from 6-19-H/L (42.8 ± 4.0%) and 6-19-L mice (44.6 ± 1.8%) than that of 6-19-H (57.5 ± 4.3%) and nontransgenic mice (60.2 ± 3.8%). Lack of differences between 6-19-H/L and 6-19-L mice suggested that the compromised development of mature B cells in the 6-19-H/L mice was likely to result from the expression of the 6-19 L chain transgene, but not from the induction of tolerance of B cells expressing IgG3 anti-IgG2a RF.

Since the 6-19 RF mAb is specific for mouse IgG2a, the spontaneous production of IgG3 anti-IgG2a RF was analyzed in these four groups of mice. The 6-19-H/L mice had high titers of IgG3 anti-IgG2a RF.
The development of glomerular lesions in 6-19-H/L, 6-19-H, and 6-19-L transgenic mice was evaluated by histological examinations for a period of 12 mo. Focal glomerular lesions with mild increases in glomerular cellularity and some polymorphonuclear leukocyte (PMN) infiltration was seen in the majority of the 6-19-H/L double-transgenic mice. These RF titers in both 6-19-H and 6-19-H/L mice remained constant from 2 to 12 mo of age (data not shown).

**Development of glomerulonephritis and necrotizing arteritis in the 6-19-H/L double-transgenic mice, but not in the 6-19-H single-transgenic mice**

The development of glomerular lesions in 6-19-H/L, 6-19-H, and 6-19-L transgenic mice was evaluated by histological examinations for a period of 12 mo. Focal glomerular lesions with mild increases in glomerular cellularity and some polymorphonuclear leukocyte (PMN) infiltration was seen in the majority of the 6-19-H/L double-transgenic mice analyzed at 2 mo of age (Fig. 2). At 4–6 mo of age, these lesions became diffuse and much more severe (Fig. 2), with increasing glomerular cellularity, inflammatory exudates, and PAS-positive deposits in mesangium and glomerular capillary walls (Fig. 3A); they resembled those observed in nontransgenic mice (Fig. 3). A comparable necrotizing arteritis with severe glomerulonephritis was seen in Wegener’s granulomatosis and microscopic angitis, in which the sera of the patients show elevated levels of fibrinoid necrosis and PMN infiltration in association with a perivascular mononuclear cell infiltration (Fig. 3G). By immunofluorescence analysis, the majority of these mononuclear cells were IgG3-positive cells and CD4+ T cells, and significant IgG3 deposits were observed in vascular walls (Fig. 4). Necrotizing arteritis was also observed in small arteries in skeletal muscle (Fig. 3H), but not in other tissues and organs, including skin, lung, liver, heart, and spleen. The incidence of this arteritis, found only in mice with the most severe glomerular lesions, was 36% (10 of 28 mice at 8–12 mo of age), although it may have been underestimated because of its focal nature. In contrast, no arteritis was observed in 6-19-H, 6-19-L, and nontransgenic mice at 8–12 mo of age.

A comparable necrotizing arteritis with severe glomerulonephritis was seen in Wegener’s granulomatosis and microscopic angitis, in which the sera of the patients show elevated levels of ANCA activities (22). Sera from the 6-19-H mice exhibited significant IgG3 ANCA activities which were however much lower in the sera of the 6-19-H/L mice (data not shown). Since purified 6-19 mAb had no ANCA activity, this observation suggests that the ANCA activity in the 6-19-H mice resulted from a combination of the 6-19 H chains with endogenous L chains. However, the lack of correlation between ANCA and necrotizing arteritis in the 6-19-H/L mice and the lack of arteritis in the 6-19-H mice appear to rule out a role for ANCA in the development of necrotizing arteritis in the 6-19-H/L mice.

**Lack of cutaneous leukocytoclastic vasculitis in the 6-19-H/L mice and its induction by raising 6-19 RF levels through i.p. transplantation of 6-19 hybridoma cells**

Normal mice injected with 6-19 hybridoma cells typically develop vascular purpura characterized by PMN infiltration and erythrocyte extravasation involving dermal small vessels of the ears, footpads, and tail (11). Despite the development of necrotizing arteritis in the 6-19-H/L mice, none of them developed cutaneous leukocytoclastic vasculitis at any age (Fig. 5A). This could be related to the relatively lower levels of 6-19 IgG3 RF present in the transgenic mice compared with hybridoma-transplanted mice. This possibility was addressed using two approaches. First, i.p. implantation of hybridoma cells on 6-19-H/L mice showed 8–10 days after implantation a 3-fold increase in serum levels of IgG3 anti-IgG2a RF, whereas RF was hardly detectable in both 6-19-L and nontransgenic control mice (Fig. 1). Substantial levels of RF were also detectable in sera from 6-19-H single-transgenic mice, but their titers were ~10 times lower than those in the 6-19-H/L double-transgenic mice. These RF titers in both 6-19-H and 6-19-H/L mice remained constant from 2 to 12 mo of age (data not shown).

**FIGURE 1.** Serum levels of total IgG3, IgG3 cryoglobulins, and IgG3 anti-IgG2a RF in 2-mo-old 6-19-H/L, 6-19-H, 6-19-L and nontransgenic (NTg) littermates. Results are expressed as milligrams per milliliter for IgG3, micrograms per milliliter for cryoglobulins, and milligrams per milliliter for RF.
RF associated with the development of cutaneous vasculitis in all seven mice (Table I and Fig. 5B). The lack of development of skin lesions in 6-19-H/L mice treated only with pristane (data not shown) ruled out the possible role of pristane priming in the generation of cutaneous leukocytoclastic vasculitis. Second, s.c. implantation of hybridoma cells on nontransgenic (MRL × BALB/c)F1 mice (a procedure which allowed serum of IgG3 anti-IgG2a RF to reach within 30–40 days levels almost comparable to those observed in the 6-19-H/L mice) showed that none of these mice developed skin vascular lesions (Table I and Fig. 5C). Notably, most of them developed glomerular lesions (Fig. 5D) similar to those seen in the 6-19-H/L mice at 4–6 mo of age and less severe than those of mice implanted i.p. with the 6-19 hybridoma cells (Fig. 5E).

Lack of the induction of glomerulonephritis by i.p. implantation of hybridoma cells secreting a hybrid 6-19H/9A6L IgG3 mAb

The development of glomerulonephritis in the 6-19-H/L, but not 6-19-H, mice suggests the possibility that the pathogenicity of IgG3 cryoglobulins may be related to the generation of large amounts of a monoclonal form (for both L and H chains) of IgG3 cryoglobulins bearing the 6-19 H chains. Therefore, we generated a hybrid 6-19H/9A6L IgG3 mAb with cryoglobulin activity but devoid of the anti-IgG2a RF activity by transfecting a 9A6 H chain loss mutant with the 6-19 H chain gene construction and assessed its pathogenicity. Intraperitoneal implantation of 6-19H/9A6L transfectoma cells into (MRL × BALB/c)F1 mice induced a rapid and remarkable increase in serum levels of IgG3 and cryoglobulins within 8 days, the levels of which were 4.5- and 2.5-fold higher, respectively, than those in mice injected i.p with 6-19 hybridoma cells (Table II). Despite this very high level of serum IgG3 cryoglobulins, none of the mice developed significant glomerular lesions (Fig. 5F), strongly arguing against the idea that the pathogenicity of IgG3 cryoglobulins bearing the 6-19 H and L chains is mainly the result of the monoclonality of these cryoglobulins.

Discussion

In the present study, we have generated transgenic mice expressing only the H chain gene or both H and L chain genes of the 6-19 IgG3 anti-IgG2a RF mAb with cryoglobulin activity and assessed the development of glomerulonephritis and vasculitis in relation to serum levels of IgG3 cryoglobulins and anti-IgG2a RF. Despite similarly increased production of IgG3 cryoglobulins in both 6-19-H and 6-19-H/L transgenic mice, only the 6-19-H/L mice expressing high levels of 6-19 IgG3 RF spontaneously developed a very severe, lethal form of chronic glomerulonephritis exhibiting very heterogeneous lesions within 8–10 mo of age; about one-third of these older mice developed in addition a focal necrotizing arteritis in the kidney and skeletal muscle. All of these changes are contrasted with the renal and vascular lesions resulting from the

![FIGURE 3. Representative histological appearance of glomerular and vascular lesions in different ages of the 6-19-H/L mice. A. A moderately severe glomerular lesion characterized by an increase of glomerular cellularity from a 4-mo-old 6-19-H/L mouse (PAS; original magnification, ×200). B–D. Severe, obliterated glomerular lesions associated with an extensive tubular cast formation (B) from 10-mo-old 6-19-H/L mice. Note crescent formation (C) or membranoproliferative changes associated with capillary wall thickening due to characteristic reduplication of glomerular basement membrane (double-contoured basement membranes; arrowheads in D) (PAS; original magnifications: B, ×100; C and D, ×200). E and F, Normal appearance of glomeruli from 12-mo-old 6-19-H and 6-19-L mice (PAS; original magnification, ×200). G and H, A severe necrotizing arteritis with fibrinoid necrosis and PMN infiltration associated with perivascular mononuclear cell infiltration in kidney (G) and skeletal muscle (H) from a 10-mo-old 6-19-H/L mouse (G: PAS; original magnification, ×200; H: H&E; original magnification, ×100).](http://www.jimmunol.org/)
rapid induction of high serum levels of the 6-19 RF mAb, as obtained within 8–10 days by implantation of the 6-19 hybridoma on normal mice. In the latter situation, the glomerular lesion consisted of massive endothelial wire loop and intracapillary deposits, and a cutaneous leukocytoclastic vasculitis rather than a renal and muscular necrotizing arteritis was observed. These data thus indicate that a unique IgV region sequence, as a result of the combination of the 6-19 H and L chains, is critical for the expression of the pathogenic activity of IgG3 cryoglobulins and that a single cryoglobulin autoantibody is capable of inducing a wide variety of glomerular lesions and different types of vascular complications, depending on its production levels and kinetics and on the duration of the cryoglobulin presence in the circulating blood.

Table I. Induction of skin vasculitis in the 6-19-H/L mice transplanted i.p. with 6-19 hybridoma cells, but not in (MRL × BALB/c)F1 mice transplanted s.c. with 6-19 hybridoma cells

<table>
<thead>
<tr>
<th>Micea</th>
<th>Cellsb</th>
<th>IgG3 RFc</th>
<th>Lesions</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Skinb</td>
</tr>
<tr>
<td>6-19-H/L</td>
<td>6-19 i.p.</td>
<td>4.6 ± 1.2</td>
<td>7/7</td>
</tr>
<tr>
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<td>None</td>
<td>1.5 ± 0.3</td>
<td>0/10</td>
</tr>
<tr>
<td>MRL × BALB/c</td>
<td>6-19 s.c.</td>
<td>1.8 ± 0.8df</td>
<td>0/15</td>
</tr>
<tr>
<td>MRL × BALB/c</td>
<td>6-19 i.p.</td>
<td>4.2 ± 1.2</td>
<td>8/8</td>
</tr>
<tr>
<td>MRL × BALB/c</td>
<td>None</td>
<td>&lt;0.01</td>
<td>0/7</td>
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</table>

a 6-19 hybridoma cells (10⁶) were inoculated i.p. or s.c. into 2-mo-old 6-19-H/L or nontransgenic (MRL × BALB/c)F1 mice. Animals were sacrificed at 8–10 days after the i.p. injection and 30–40 days after the s.c. injection for serological and histological analysis. Serum levels of IgG3 anti-IgG2a RF (milligram per milliliter) were determined at sacrifice (mean of 7–15 mice ± 1 SD).

b Incidence of macroscopically visible skin lesions.

c Semiquantitative values of glomerular lesions on a 0–4+ scale based on the intensity of lesions are indicated (mean ± 1 SD).

d Serum IgG3 RF became detectable 3 days after i.p. injection of hybridoma cells and rapidly reached higher levels until 8–10 days. In contrast, IgG3 RF became detectable in sera 8 days after s.c. implantation of hybridoma cells and slowly increased thereafter. Serum concentrations of IgG3 RF at 30–40 days after s.c. injection of hybridoma cells were comparable to those at 5 days after i.p. hybridoma injection.

Serological analysis in the 6-19 transgenic mice has shown that the 6-19-H/L mice produced increased amounts of IgG3 cryoglobulins at levels comparable to those in the 6-19 H chain gene-alone transgenic mice, despite their differences in L chain composition of transgenic IgG3 Abs. Nevertheless, only the 6-19-H/L mice having ~10-fold higher levels of IgG3 anti-IgG2a RF than the 6-19-H mice spontaneously developed a severe, lethal form of glomerulonephritis. This indicates that the majority of IgG3 cryoglobulins resulting from a heterogeneous combination of the 6-19 H chains with endogenous L chains in the 6-19-H mice are poorly nephritogenic, and that the 6-19-H mice acquire the nephritogenic activity only when combined with appropriate L chains. This notion is consistent with the fact that only a fraction of IgG3 monoclonal cryoglobulins is able to provoke significant glomerular lesions after implantation of the relevant hybridomas (11, 21, 23–26), and that the nephritogenicity of the 6-19 mAb was conserved with 6-19H/J558L hybrid mAb which has no RF activity (13), but not with the 6-19H/9A6L IgG3 hybrid mAb.

Our present demonstration that quantitative differences of IgG3 6-19 RF in 6-19-H/L and 6-19-H mice critically determine the presence and absence of glomerulonephritis strongly argues for the potential role of the ligand-binding property of the 6-19 RF mAb in the generation of glomerular lesions. We have previously demonstrated that Ig-deficient mice are still able to develop typical

Table II. Lack of the development of glomerular lesions in mice implanted with transfectoma-secreting 6-19H/9A6L hybrid IgG3 mAb

<table>
<thead>
<tr>
<th>mAb</th>
<th>IgG3a</th>
<th>Cryoglobulina</th>
<th>Kidneyb</th>
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<tbody>
<tr>
<td>6-19H/9A6L</td>
<td>18.6 ± 2.4</td>
<td>93.6 ± 14.0</td>
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</tr>
<tr>
<td>6-19</td>
<td>4.3 ± 1.0</td>
<td>37.6 ± 12.6</td>
<td>7/7</td>
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a 6-19H/9A6L transfectoma (10⁶) or 6-19 hybridoma cells were inoculated i.p. into 2-mo-old (MRL × BALB/c)F1 mice. Mice were sacrificed 8–10 days later for serological and histological analysis. Serum levels of total IgG3 (milligram per milliliter) and cryoglobulins (micrograms per milliliter) were determined at sacrifice (mean of seven to eight mice ± 1 SD).

b Incidence of glomerular lesions.
glomerular lesions following the injection of 6-19 hybridoma cells (13, 14). Thus, it was concluded that the IgG3 RF-IgG2a immune complex formation is not required for the induction of glomerulonephritis in this model. A possible explanation may be that the 6-19 RF mAb could exhibit an additional ligand-binding property other than the RF activity thereby involved in the development of glomerulonephritis; dual specificity of autoantibodies, such as anti-DNA and RF, has been repeatedly demonstrated (27–30). In this regard, we have observed in preliminary experiments a definite binding in vitro of the 6-19 mAb to cultured mesangial cells, suggesting the possibility of a local glomerular ligand for this mAb in vivo. An alternative possibility we cannot totally exclude is that the nephritogenic potential of at least some IgG3 cryoglobulins might be determined by physicochemical properties, probably related to the V-region amino acid sequences, irrespective of their Ag-binding specificity.

With respect to vascular lesions induced by the 6-19 mAb, the 6-19-H/L transgenic mice do not have spontaneous cutaneous leukocytoclastic vasculitis typically seen in mice implanted with 6-19 hybridoma cells, but develop with age focal necrotizing arteritis in kidneys and skeletal muscles. Differences in circulating levels of 6-19 RF mAb and in their production kinetics apparently account for the development of different vascular lesions between the 6-19 H/L mice and 6-19 hybridoma-implanted mice. The skin vasculitis requires RF activity (13, 14) and results from the activation of mast cells through the interaction of IgG Fc receptors with RF immune complexes triggering an inflammatory reaction and PMN recruitment (31). It appears that the concentration of RF in the 6-19 H/L mice is not high enough to generate sufficient amounts of RF immune complexes, since when it is increased in transgenic mice by hybridoma implantation, skin lesions do occur, while when it is kept lower with hybridoma as the result of their s.c. rather than i.p. implantation, skin lesions do not occur. Thus, cutaneous leukocytoclastic vasculitis is an acute inflammatory lesion associated with a rapid elevation of serum concentrations of the 6-19 RF cryoglobulins. In contrast, focal necrotizing arteritis is likely to be related to the chronic presence of pathogenic 6-19 IgG3 RF cryoglobulins. Notably, among several different lupus-prone mice, MRL-fpr/lpr mice, which spontaneously generate remarkably high levels of IgG3 cryoglobulins with RF activity (8, 32, 33), is the only strain that develops similar necrotizing arteritis (32, 34–37). We observed that the 6-19 H chains are capable of conferring an ANCA activity in combination with endogenous L chains. However, the absence of any vascular lesions in the 6-19 H mice having higher titers of ANCA excludes the role of ANCA in the pathogenesis of necrotizing arteritis occurring in the 6-19-H/L mice.

The 6-19-H/L transgenic mice established in the present study provide a new experimental model of necrotizing arteritis as well as chronic glomerulonephritis associated with cryoglobulinemia, the pathogenesis of which has been poorly understood so far. In addition, we have observed in preliminary studies a contrasting incidence of glomerular lesions in the 6-19-H/L mice backcrossed with BALB/c and DBA/2 mice, respectively, low and high. This suggests that the nephritogenicity of 6-19 IgG3 cryoglobulins is controlled by genetic factor(s), which act by affecting glomerular localization of pathogenic autoantibodies or subsequent inflammatory responses to deposited autoantibodies. Clearly, this experimental model would allow us to better define pathogenic and genetic mechanisms leading to the spontaneous development of glomerular and vascular lesions as a consequence of the persistent presence of autoantibodies with cryoglobulin activity. A better understanding of molecular and cellular events involved in the generation of cryogenic autoantibody-mediated tissue lesions could have promising clinical implications in the design of future diagnostic and therapeutic strategies.

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References


