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# Cryoglobulinemia induced by a murine IgG3 rheumatoid factor: Skin vasculitis and glomerulonephritis arise from distinct pathogenic mechanisms

(autoantibody/hybrid antibody/MRL-*lpr/lpr*/variable region sequence/systemic lupus erythematosus)

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ABSTRACT **MRL-*lpr/lpr* mice spontaneously develop a lupus-like syndrome characterized by immunopathological manifestations such as necrotizing vascular lesions of ear tips and severe glomerulonephritis. Similar skin vascular and glomerular lesions associated with cryoglobulinemia can be induced in normal mice by injection of a monoclonal antibody (mAb)—6-19 (γ3 heavy chain and κ light chain), exhibiting both cryoglobulin and anti-IgG2a rheumatoid factor (RF) activities—derived from the MRL-*lpr/lpr* autoimmune mouse. To determine the role of RF and/or IgG3 $F_c$ fragment-associated cryoglobulin activities in 6-19 mAb-induced tissue lesions, a 6-19–J558L hybrid mAb (γ3 heavy chain and λ1 light chain) was produced by fusion between the 6-19 hybridoma and the J558L myeloma. Here we report that the 6-19–J558L hybrid mAb, which loses the RF activity but retains the cryoglobulin activity, fails to induce skin vascular lesions. However, it is still able to provoke glomerular lesions identical to those caused by the 6-19 mAb. Further, we have observed that the depletion of the corresponding autoantigen, IgG2a, in mice by treatment with anti-IgM antisera from birth also prevents the development of skin but not glomerular lesions. Our results indicate that both RF and cryoglobulin activities of the 6-19 mAb are required for the development of skin vasculitis, but its cryoglobulin activity alone is sufficient to cause glomerular lesions. In addition, cDNA cloning and sequencing of the 6-19 mAb has revealed that the 6-19 κ light chain variable region amino acid sequence is encoded in a germ-line configuration, suggesting that immunoglobulin variable region germline genes could contribute to the generation of pathogenic autoantibodies.**

MRL-*lpr/lpr* autoimmune mice spontaneously develop pathologic abnormalities, such as arthritic-like lesions, necrotizing vascular lesions of the skin of ears and foot pads, and severe glomerulonephritis, similar to those found in human patients with systemic lupus erythematosus and rheumatoid arthritis (1). Serologically, they develop high titers of IgG anti-IgG rheumatoid factor (RF) autoantibodies and remarkably high concentrations of cryoglobulins (1–3). However, the precise role of RF and cryoglobulins in the pathogenesis of vascular and glomerular lesions has been poorly understood.

To determine the role of RF and cryoglobulins in the pathogenesis of vascular and glomerular lesions, we recently investigated the pathogenic effects of a panel of anti-IgG2a RF monoclonal antibodies (mAbs), obtained from unimmunized MRL-*lpr/lpr* mice, in normal strains of mice. It was found that only IgG3 anti-IgG2a RF mAb, which is able to generate cryoglobulins as a result of IgG3 $F_c$–$F_c$ interaction, induced extensive pathological manifestations including peripheral vasculitis and glomerulonephritis (ref. 4; T.B., A. Marshak-Rothstein, and S.I., unpublished data). However, the respective contributions of RF and cryoglobulin activities of the IgG3 anti-IgG2a mAb to the development of the two types of tissue lesions have not been defined. In the present study using an IgG3 anti-IgG2a RF mAb, clone 6-19 (4), the role of RF in IgG3 RF cryoglobulin-associated pathology was investigated by suppression of the RF activity of 6-19 mAb and by B-cell depletion of 6-19 mAb-recipient mice. Results presented here indicate that the RF autoantibody activity, in association with the cryoglobulin formation, critically contributes to the development of skin vascular lesions, but the cryoglobulin activity alone is sufficient to induce the glomerular lesions.

## MATERIALS AND METHODS

**Mice and Their Treatment.** BALB/c mice were obtained from Bomholtgard (Ry, Denmark), and MRL-+/+ mice were from The Jackson Laboratories. Their $F_1$ hybrid mice were bred in our own facilities. The induction and maintenance of B-cell suppression were performed as described (5). Briefly, newborn BALB/c mice were injected with 0.1 ml of rabbit anti-IgM antiserum i.p. on days 1, 2, 3, 5, 7, and 9 and then with 0.3 ml of anti-IgM antisera twice a week. Anti-IgM-treated adult BALB/c females were then mated with MRL-+/+ males, and treatment was continued throughout pregnancy and lactation. Newborn litters were treated as described above. Control mice were similarly treated with normal rabbit serum (NRS).

**mAb.** The 6-19 mAb [γ3 heavy (H) chain and κ light (L) chain] and 2-6D mAb (γ3, κ) were obtained by fusion of spleen cells from unmanipulated MRL-*lpr/lpr* mice as described (4). An IgG2b anti-6-19 anti-idiotypic (anti-Id) mAb was prepared as described (6). The IgG3 6-19–J558L hybrid mAb, clone L8D, was established by fusion of the 6-19 hybridoma with the J558L myeloma secreting J558 λ1 L chains (7). Rat anti-mouse κ-chain mAb, H139.52.1 (8); rat anti-mouse γ3-chain mAb, H139.61.1 (8); mouse anti-mouse λ1-chain mAb LS 136 (9); mouse anti-trinitrophenyl (TNP), Hy 1.2 (10); and mouse anti-rat κ-chain mAb (MARK-1) (11) were provided by M. Pierres (Marseille, France), K. Rajewsky (Cologne, F.R.G.), M. Nose (Sendai, Japan), and H. Bazin (Brussels), respectively. Murine and rat mAbs were

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Abbreviations: AMG, aggregated mouse IgG; Id, idiotype (idiotypic); mAb, monoclonal antibody; NRS, normal rabbit serum; RF, rheumatoid factor; H, heavy; L, light; $V_H$ and $V_L$, variable region of the H and L chains; D, diversity; J, joining; C, constant; PAS, periodic acid/Schiff reagent.

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purified from culture supernatants by protein A and MARK-I affinity column chromatography (11).

**ELISA.** Supernatants of hybridoma cells were screened by ELISA for reactivity with anti-κ, anti-λ1, and anti-6-19 anti-Id reagents. Culture supernatants or purified mAb were added to microtiter wells coated with rat anti-mouse γ3 mAb. The assays were developed with anti-λ1, anti-κ, or anti-6-19 anti-Id mAb conjugated with alkaline phosphatase. Results are expressed as the OD value at 405 nm. IgG2a and IgG3 concentrations in sera or cryoglobulins were quantitated by ELISA as described (12).

**RF Assay.** $^{125}$I-labeled aggregated mouse IgG ($^{125}$I-AMG) was prepared by heating myeloma protein, MOPC21 (IgG1), UPC10 (IgG2a), MOPC195 (IgG2b), or J606 (IgG3) at 0.5 mg/ml, mixed with trace amounts of radiolabeled corresponding IgG, at 63°C for 30 min and was diluted to 0.1 mg/ml in 0.01 M phosphate-buffered saline (pH 7.2) containing 0.05% Tween 20. RF activities of purified mAb or sera were measured by the precipitation of $^{125}$I-AMG at 4°C overnight as described (4). $^{125}$I-AMG bound to IgG RF was precipitated by centrifugation at 1880 × *g* for 5 min. The results are expressed as a percentage of $^{125}$I-AMG precipitated. In some experiments, the anti-IgG2a RF activity was determined by ELISA as described by Wolfowicz *et al.* (13). Briefly, microtiter plates were coated with TNP-conjugated bovine serum albumin and subsequently were incubated with IgG2a anti-TNP mAb, Hy 1.2. After an overnight incubation with various amounts of mAb, the assay was developed with anti-mouse γ3 mAb conjugated with alkaline phosphatase.

**cDNA Cloning and Sequencing.** RNA was prepared from 6-19 hybridoma by the LiCl method, and poly(A)$^+$ RNA was isolated on an oligo(dT)-cellulose column as described (14). Double-stranded cDNA was synthesized by the method of Gubler and Hoffman (15) with an oligo(dT) primer and 2.5 μg of poly(A)$^+$ RNA. The double-stranded cDNA was inserted either in the oligo(dC)-tailed *Pst* I site of the plasmid pUC19 after oligo(dG)-tailing for H-chain cDNA cloning or in the oligo(dG)-tailed *Pst* I site of the plasmid pUC19 after oligo(dC)-tailing for L-chain cDNA cloning. The library was screened with a 1.5-kilobase (kb) *Bam*HI-digested DNA fragment isolated from plasmid pJ558 (16) and a 1.7-kb *Hin*dIII/*Xba* I-digested DNA fragment isolated from plasmid pJκ1-5 (17), respectively provided by C. Paige (Toronto) and K. Rajewsky. The probes were radiolabeled to a specific activity of 2 × 10$^8$ cpm/μg by using a multiprime DNA labeling system and [α-$^{32}$P]dCTP (Amersham). The nucleotide sequences corresponding to the variable regions of the H and κ chains ($V_H$ and $V_K$) were determined† by the dideoxynucleotide chain termination method (18) using the Sequenase sequencing system (United States Biochemical) with deoxyadenosine 5′-[α-($^{35}$S)thio]triphosphate. Oligonucleotide primers were as follows (5′ to 3′): phage M13 sequencing primer (GTAAAACGACGGCCAGT), M13 reverse-sequencing primer (AACAGCTATGACCATG), γ3-chain constant region gene ($C\gamma_3$) primer (GGATAGACAGATGG) complementary to codons 119–123, and 5′ UT $V_H$ reverse-sequencing primer (CACTGACTTTCACCATG) for the H-chain cDNA clone and with the help of $C_\kappa$ primer (TGGATGGTGGGAAGATG) (116–122) for the L-chain cDNA clone. Oligonucleotide primers were synthesized with a system 1 Beckman DNA synthesizer.

## RESULTS

**Generation and Characterization of an IgG3 6-19–J558L Hybrid mAb, Clone L8D.** Following fusion of 6-19 hybridoma cells with the J558L myeloma, hybridomas secreting hybrid mAb bearing the λ1 light chain from J558L myeloma cell and the IgG3 H chain from 6-19 hybridoma were selected by the ability of culture supernatants to bind in a first step to rat anti-mouse γ3 mAb coated on microtiter plates and in a second step to be recognized by mouse anti-λ1 or rat anti-κ mAb. One of the resulting hybrid antibodies, clone L8D, was detected by the anti-λ1 antibody but not by the anti-κ antibody (Fig. 1 *A* and *B*), whereas 6-19 mAb exhibited no significant binding to the anti-λ1 antibody. In addition, when the reactivity of the L8D mAb with the anti-6-19 anti-Id mAb was analyzed, no binding was observed with L8D mAb (Fig. 1*C*).

To determine the effect of L-chain replacement on the anti-IgG2a RF activity, increasing amounts of L8D mAb were incubated with $^{125}$I-radiolabeled IgG2a aggregates (AMG) overnight at 4°C prior to centrifugation. While 6-19 mAb precipitated $^{125}$I-AMG in a dose-dependent manner, L8D mAb failed to precipitate significant amounts of $^{125}$I-AMG (Fig. 1*D*), indicating that the hybrid L8D mAb had lost the IgG2a binding specificity. Notably, L8D mAb exhibited no RF activity against any other IgG subclass. The lack of anti-IgG2a RF activity of the L8D mAb was further confirmed by a solid-phase RF assay (data not shown). In contrast, the L8D mAb was still able to generate cryoglobulins, although exchange of the 6-19 κ L chain by the J558L λ1 L chain substantially reduced the cryoglobulin activity. When 1 ml of purified L8D or 6-19 mAb (1 mg/ml) were incubated at 4°C for 2 days, approximately one-fifth of the cryoprecipitates were recovered after centrifugation of L8D mAb (40 μg) as compared with 6-19 mAb (190 μg). Notably, no significant cryoprecipitation (<1 μg) was obtained with a noncryoprecipitable IgG3 2-6D mAb.

**Induction of Glomerulonephritis but not Skin Vasculitis by L8D Hybridoma.** Since the L8D hybrid mAb was found to lack the anti-IgG2a RF activity but still retain significant cryoglobulin activity, we studied whether the lack of RF autoantibody activity could affect the pathogenic activities. (MRL × BALB/c)$F_1$ mice injected with L8D hybridoma cells failed to develop the characteristic vascular purpura on the skin of their ears as observed with the 6-19 hybridoma (Table 1; Fig. 2 *A* and *B*). Notably, periodic acid/Schiff reagent (PAS)-positive substances, most likely cryoglobulins, were often found in the capillary lumen without apparent inflam-

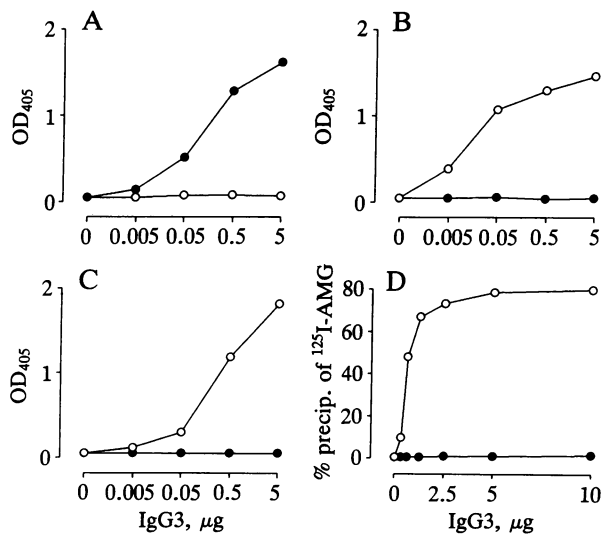


FIG. 1. Expression of λ1 L chain (*A*), κ L chain (*B*), 6-19 Id (*C*), and anti-IgG2a RF activity (*D*) in 6-19 (○) and L8D (●) mAbs. Results are expressed as OD at 405 nM (*A*, *B*, and C) or as the percent of precipitation (precip.) of $^{125}$I-AMG (*D*).

†The $V_H$ and $V_L$ sequences reported here have been deposited in the GenBank data base (accession nos. M55312 and M55313, respectively).

Table 1. *In vivo* effects of 6-19 L-chain exchange and B-cell depletion on the development of skin vasculitis and glomerulonephritis

| Hybridoma | Pretreatment* | IgG2a,† mg/ml | IgG3,† mg/ml | Cryogloblins,† μg/ml | RF,† % | Skin lesions | Kidney lesions |
|---|---|---|---|---|---|---|---|
| L8D | None | 1.5 ± 0.4 | 5.2 ± 0.3 | 25 ± 6 | 0.6 ± 0.2 | 0/8 | 8/8 |
| 6-19 | Anti-IgM | <0.1 | 5.7 ± 0.8 | 148 ± 90 | 40.7 ± 4.8 | 0/5 | 5/5 |
| 6-19 | NRS | 1.8 ± 0.4 | 5.6 ± 0.4 | 97 ± 22 | 44.9 ± 6.2 | 10/10 | 10/10 |

Hybridoma cells ($10^7$) were inoculated i.p. into (MRL × BALB/c)$F_1$ mice. Animals were sacrificed between 10 and 15 days later for serological and histological analysis.

*(MRL × BALB/c)$F_1$ mice were treated from birth with either rabbit anti-mouse IgM antisera or NRS. When 6-19 hybridoma cells were injected into (MRL × BALB/c)$F_1$ mice, results were essentially identical to those of the NRS-pretreatment group (data not shown).

†Serum levels of IgG2a, IgG3, and cryoglobulins were quantitated by ELISA (15); and IgG2a-binding RF activities of 1 μl of serum, by RIA (5). IgG2a levels were determined prior to hybridoma injection; and others, at sacrifice.

matory reaction (Fig. 2*C*). In contrast, all mice injected with L8D as well as 6-19 hybridoma developed a severe acute glomerulonephritis as documented by polymorphonuclear neutrophil infiltration and marked deposits of PAS-positive materials along the glomerular capillary walls as well as in capillary lumens (Fig. 2*D*). It should be mentioned that serum levels of IgG3 were comparable in both groups of mice, yet no RF activity was detectable in sera from mice injected with the L8D hybridoma (Table 1). Amounts of cryoglobulins recovered from sera of mice receiving the L8D hybridoma were one-fourth that of mice receiving the 6-19 hybridoma.

**Development of Glomerulonephritis but not Skin Vasculitis in B Cell-Depleted Mice Injected with 6-19 Hybridoma.** To study further the role of the anti-IgG2a RF activity on the development of 6-19 RF cryoglobulin-associated tissue lesions, (BALB/c × MRL)$F_1$ recipient mice were depleted of B cells by treatment from birth with rabbit anti-mouse IgM antiserum, which markedly reduced serum levels of IgG2a (Table 1). Although serum levels of IgG3, cryoglobulins, and RF in B cell-depleted mice after the 6-19 hybridoma injection did not significantly differ from control NRS-treated mice, the development of skin vascular purpura was completely inhibited in

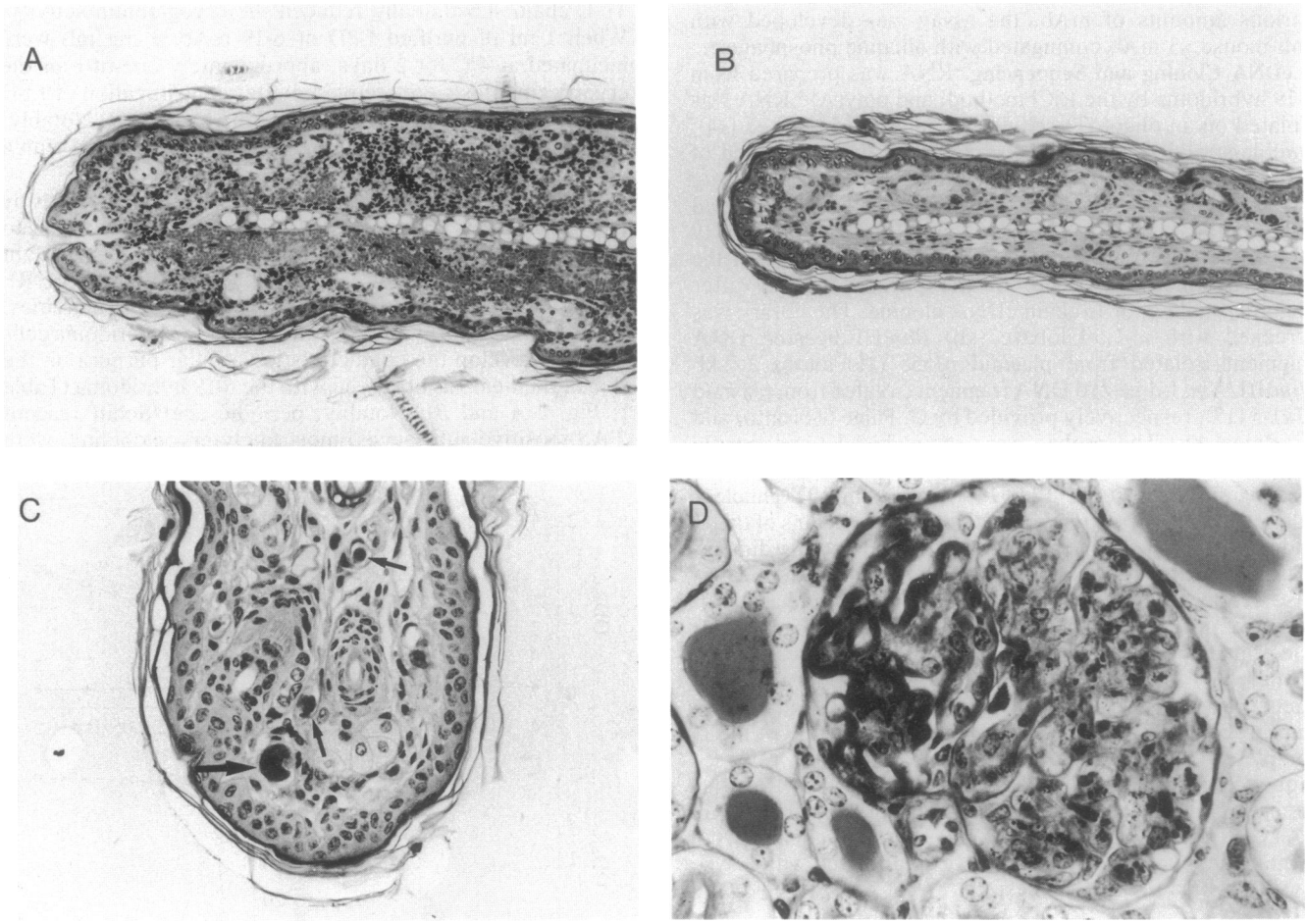


FIG. 2. (*A*) Representative histological appearance of skin lesions of ears 7 days after the i.p. injection of 6-19 IgG3 RF hybridoma cells into (MRL × BALB/c)$F_1$ hybrid mice. Leukoclastic vasculitis was characterized by the infiltration of polymorphonuclear leukocytes and the extravasation of erythrocytes. (Hematoxylin/eosin; ×60.) (*B*) Representative histological appearance of skin of ears from mice injected with a hybridoma secreting the L8D mAb. Note the absence of leukoclastic vascular lesions. (Hematoxylin/eosin; ×60.) (*C*) The presence of intracapillary precipitation of PAS-positive materials (arrows) in the skin of ears from mice injected with a hybridoma secreting the L8D mAb. Note the absence of any inflammatory reaction. (PAS; × 120.) (*D*) Representative histological appearance of glomerular lesions induced by the L8D mAb showing the proliferative and exudative changes and the voluminous deposition of PAS-positive materials along the glomerular capillary walls (PAS; ×240.)

all five mice pretreated with anti-IgM antisera. However, these mice developed a severe acute glomerulonephritis similar to control NRS-treated mice. It should be mentioned that a single transfer of 2 ml of ascites (10 mg of 6-19 mAb) recovered from these anti-IgM-treated mice into untreated BALB/c mice induced skin vascular lesions within 12 hr.

**Cloning of 6-19 $V_H$ and $V_\kappa$ Regions and DNA Sequences.** In an attempt to determine the contribution of somatic mutations in the pathogenic activity of 6-19 mAb, we performed cDNA cloning and DNA sequencing of 6-19 $V_H$ and $V_\kappa$ regions. The 6-19 $V_H$ gene (Fig. 3*A*) belongs to the J558 $V_H$ gene family, as its nucleotide sequence was found to be >80% homologous to members of the J558 $V_H$ gene family (16). However, none of the known germ-line J558 $V_H$ genes was found to be identical to the 6-19 $V_H$ genes. A comparison with an IgG2b anti-IgG2a RF mAb of MRL-*lpr/lpr* origin, clone AM11, showed the highest sequence homology (97.3%), as it differs from it by eight nucleotides only (19). The diversity region gene (*D*) sequence of 6-19 mAb differs from the BALB/c DFL16.1 germ-line gene by five nucleotide substitutions in the 23-base-pair (bp)-long common sequence (20). The H-chain joining ($J_H$) segment expressed by 6-19 mAb is identical to the BALB/c $J_H2$ sequence and also to a $J_H2$ consensus sequence derived from the analysis of several MRL hybridomas (21), suggesting that it is expressed in the germ-line configuration.

Comparison of the nucleotide sequence of $V_\kappa$ 6-19 (Fig. 3*B*) with germ-line genes in the $V_\kappa1$ family showed that $V_\kappa$ 6-19 is closely related to the $V_\kappa$1A5 germ-line gene of BALB/c origin (22). The only 2-bp differences were found at the splice site of the leader peptide at codon −4 and at the third base of codon 95. This latter substitution is likely to result from the flexibility of the junction of $V_\kappa$ and $J_\kappa$ segments during genomic rearrangement (24). This base substitution does not change, however, the amino acid residue proline. The $J_\kappa$ segment used by 6-19 mAb corresponds to the MRL/Mp $J_\kappa2$ germ-line sequence, derived from a $J_\kappa2$ consensus sequence (21).

## DISCUSSION

The molecular mechanisms of cryoglobulin-associated pathology induced by an IgG3 anti-IgG2a RF mAb, clone 6-19, derived from the MRL-*lpr/lpr* autoimmune mouse, were investigated. Here we show that the replacement of the κ L chain of this IgG3 RF mAb by the J558 λ1 L chain abolishes the anti-IgG2a RF activity but not the cryoglobulin activity. *In vivo*, the loss of the RF activity by L chain exchange results in the complete suppression of skin vasculitis, while it has no effect on the development of the glomerulonephritis. In addition, we demonstrate that the depletion of B cells in recipient mice prevents the development of 6-19 mAb-induced skin lesions but not of renal lesions. Taken together, these experiments strongly suggest that the RF activity of 6-19 mAb in association with the IgG3 cryoglobulin activity plays a critical role in the development of skin vasculitis, while the nephritogenic activity is contributed by the cryoglobulin activity alone. Dissociation of skin vascular and glomerular lesions indicates that different pathologic mechanisms govern the development of each kind of tissue lesion induced by RF cryoglobulins.

Elucidation of the molecular mechanism of the 6-19 mAb cryoglobulin- and RF-induced tissue lesions first required understanding of the importance of each of its two biological activities. The strategy used involved two methods: the first abrogates the RF activity of the 6-19 mAb, and the second depletes the corresponding autoantigen, IgG2a, from the blood circulation in 6-19 mAb-recipient mice. The effect of the loss of IgG2a binding activity of 6-19 mAb on the development of skin vasculitis by L-chain exchange and the effect of immunoglobulin depletion in 6-19 mAb-recipient mice clearly demonstrate that the RF activity of the antibody is involved in this pathology. These observations provide direct evidence that *in vivo* IgG binding of RF autoantibodies contributes to the development of an autoimmune pathology. The conclusion that both RF and cryoglobulin activities of 6-19 mAb are required to induce cutaneous vascular lesions is further supported by our recent observations (T.B., A. Marshak-Rothstein, and S.I., unpublished data): (*i*) four

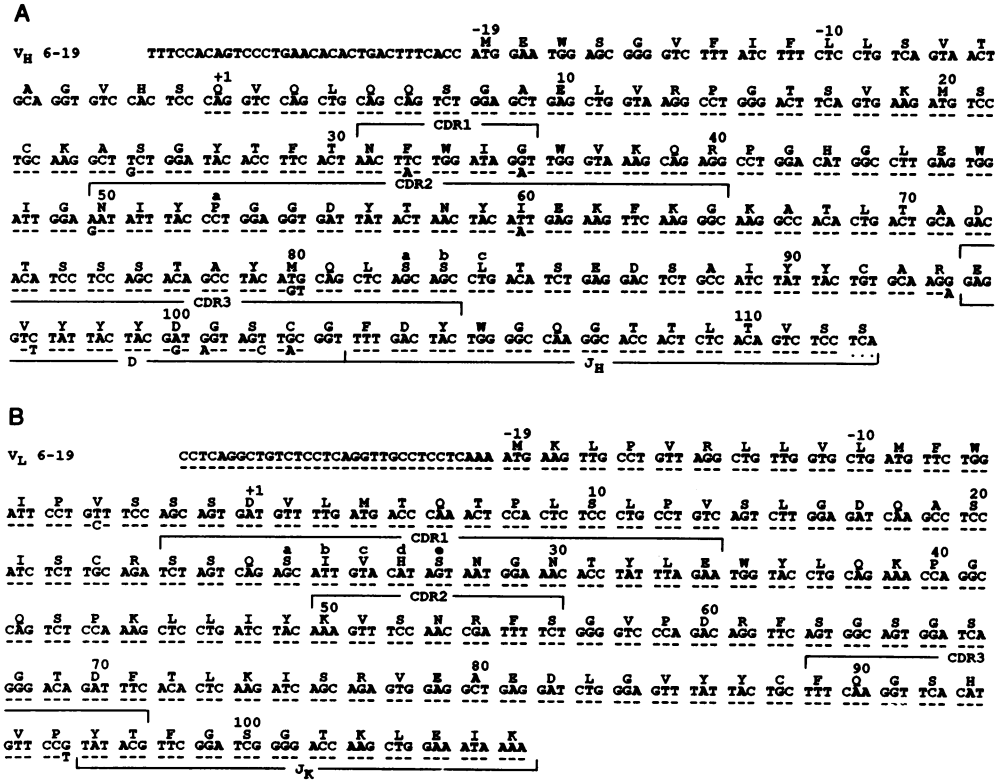


FIG. 3. Nucleotide and predicted amino acid sequences of $V_H$ region (*A*) and $V_L$ region (*B*) of 6-19 mAb. The numbering of amino acid residues and complementarity-determining regions (CDR) are according to Kabat *et al.* (23). cDNA corresponding to the mAbs were cloned, and the DNA sequences were determined. The nucleotide sequence of $V_H$ 6-19 is compared to the $V_H$ region AM11 mAb derived from the MRL-*lpr/lpr* mouse (19), to the *D* region to DFL16.1 germ-line gene of BALB/c origin (20), and to the $J_H$ segment to a $J_H2$ consensus sequence of several independent mAb obtained from the MRL-*lpr/lpr* mouse (21). The nucleotide sequence of $V_L$ 6-19 is compared to the $V_\kappa$1A5 germ-line gene of BALB/c origin (22) and to a $J_\kappa2$ consensus sequence of several independent mAb derived from the MRL-*lpr/lpr* mouse (21). Identities are indicated by dashes.

other IgG3 cryoglobulins with anti-IgG2a RF specificity also exhibited the ability to induce skin vascular lesions similar to those induced by 6-19 mAb, (*ii*) IgG1 class-switch variant of the 6-19 mAb neither generated cryoglobulins nor induced tissue lesions, and (*iii*) no IgM or IgG anti-IgG2a RF mAb of the other subclasses, lacking the cryoglobulin activity, were able to cause skin vascular lesions and glomerulonephritis.

In contrast, the development of glomerulonephritis is not affected at all by the loss of the anti-IgG2a autoantibody activity or by a marked depletion of serum IgG2a in 6-19 mAb-recipient mice. These results indicate that unlike the skin vascular lesions, the immune complex formation of 6-19 mAb with host IgG2a does not appear to be involved in the development of glomerulonephritis and that its cryoglobulin activity by itself is sufficient. However, it should be noted that not all IgG3 cryoglobulins lacking RF activity were able to induce glomerular lesions (4). Since the $F_{ab}$ region, most likely V-region sequences, could markedly influence the cryoglobulin activity of self-associating IgG3 aggregates (25), unique V-region sequences associated with anti-IgG2a RF activity may play a critical role in the nephritogenic activity of IgG3 cryoglobulins.

Much attention has been paid recently to the role of somatic mutations in the generation of autoantibodies characteristic of systemic lupus erythematosus. Although autoantibodies, particularly of the IgM class, can be entirely encoded by germ-line *V* genes (26), the frequent presence of somatic mutations among IgG autoantibodies (19, 21), which may correlate with the production of high-affinity autoantibodies, has suggested that the pathogenicity of autoantibodies may be created by somatic diversification of the germ-line repertoire. In this regard, it is worth noting that the 6-19 $V_\kappa$ region amino acid sequence is encoded in a germ-line configuration and contributes to the RF activity and therefore to the development of skin vasculitis. This indicates that immunoglobulin V-region germ-line genes (*V*) are likely involved in the generation of pathogenic autoantibodies. Obviously, this does not exclude the role of the somatic mutations, which may be present in the 6-19 $V_H$ region, in the pathogenic activity of the 6-19 mAb. In fact, the presence of five nucleotide substitutions in the 6-19 *D* region is suggestive for the presence of somatic mutations in $V_H$ 6-19. Until the germ-line counterpart of $V_H$ 6-19 is cloned, we cannot definitely address the contribution of somatic mutations to the pathogenic activity of 6-19 mAb.

The data presented here have demonstrated that two distinct pathogenic mechanisms govern the development of 6-19 RF cryoglobulin-associated cutaneous and glomerular lesions: the skin leukoclastic vasculitis is mediated by autoantigen–autoantibody immune complexes with the cryoglobulin activity of autoantibodies, and the glomerulonephritis is induced by the direct deposition of IgG3 cryoglobulins. Our recent studies on a panel of anti-IgG2a RF mAb strongly suggest that autoantibody activities by themselves may not be sufficient to provoke tissue injuries and that the cryoglobulin activity associated with the IgG3 C region plays a critical role for the pathogenic potential of autoantibodies. In this regard, it should be mentioned that the immune complex formation between the 6-19 RF mAb and anti-6-19 anti-Id mAb prevented the development of 6-19 mAb-induced skin and glomerular lesions, as a result of a rapid clearance of 6-19 RF mAb from the blood circulation and of inhibition of 6-19 mAb cryoprecipitation (6). In addition, we have recently demonstrated that among several strains of mice bearing the *lpr* gene, the spontaneous production of IgG3 RF with cryoglobulin activity was only found in MRL-*lpr/lpr* mice, which develop severe systemic disease, but not in C3H- and C57BL/6-*lpr/lpr* mice, which develop only limited tissue lesions (T.S., F.S., T.B., and S.I., unpublished data). Since human IgG3 also exhibits a physicochemical property similar to that of murine IgG3 (27, 28), our demonstration of the remarkable pathogenic activity of murine IgG3 RF cryoglobulins suggests the importance of IgG3 autoantibodies in the pathogenesis of rheumatoid arthritis, systemic lupus erythematosus, and related rheumatic diseases.

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