Molecular and Cellular Basis for Pathogenicity of Autoantibodies

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IZUI, S., BERNEY, T., SHIBATA, T., FULPIUS, T., FOSSATI, L. and MERINO, R. Molecular and Cellular Basis for Pathogenicity of Autoantibodies. Tohoku J. Exp. Med., 1994, 173 (1), 15-30 —— Using two different kinds of monoclonal autoantibodies, anti-mouse RBC (MRBC) autoantibodies and IgG3 rheumatoid factor (RF) cryoglobulins, we have attempted to better define the molecular and cellular basis of the pathogenicity of autoantibodies. Among eight anti-MRBC monoclonal antibodies (mAbs) obtained from NZB mice, only five of them are able to cause anemia. The distinct differences in specificity between pathogenic and non-pathogenic anti-MRBC mAbs emphasize the importance of autoantibody specificity for the pathogenesis of autoimmune hemolytic anemia. Histological examination has revealed that Fcγ receptor-mediated erythrophagocytosis and sequestration of agglutinated RBC in spleens and livers are the major pathogenic mechanisms of hemolytic anemia. This indicates that the affinity of autoantibodies for the Fcγ receptors of phagocytes and/or the ability to cause hemagglutination, both of which vary among immunoglobulin isotypes, are additional factors determining the pathogenic activity of anti-MRBC autoantibodies. Studies on a panel of anti-IgG2a RF mAbs derived from MRL-lpr/lpr mice have demonstrated that only the IgG3 isotypes of RF mAb are able to generate cryoglobulins and to induce skin leukocytoclastic vasculitis and glomerulonephritis in normal mice. Although the cryoglobulin activity of RF mAb associated with the IgG3 isotype has been shown to be solely responsible for the generation of glomerular lesions (both RF and cryoglobulin activities are necessary for cutaneous vascular lesions), the absence of nephritogenic activity by some IgG3 monoclonal cryoglobulins supports the idea that qualitative features of cryoglobulins are critical to determine their pathogenic activities. Of interest, IgG3 autoantibodies lacking the cryoglobulin activity may not be harmful, but even protective against the development of IgG3 cryoglobulin-mediated tissue lesions, because they inhibit the cryoglobulin formation of pathogenic IgG3 autoantibodies as a result of their nonspecific IgG3 Fc-Fc interaction. Our results on monoclonal autoantibodies clearly indicate the importance of certain subpopulations of autoantibodies in the pathogenesis of autoantibody-mediated cellular and tissue injuries. ——— autoantibody; autoimmune hemolytic anemia; cryoglobulin; glomerulonephritis;
Systemic lupus erythematosus (SLE) is one of a group of diseases of unknown etiology associated with evidence of autoimmune responses. It is characterized by the formation of a variety of autoantibodies reactive to self or altered self antigens. The direct binding of certain autoantibodies such as anti-erythrocyte autoantibodies could cause autoimmune cellular damage. Other autoantibodies such as anti-DNA antibodies could provoke tissue lesions as a result of their deposition in glomeruli and small vessels. In fact, glomerulonephritis induced by the deposition of such autoantibodies, either through the formation of immune complexes with corresponding autoantigens, the direct interaction with glomerular structures or other not yet well-defined mechanisms, is the major cause of death in patients with SLE.

It is clear that autoantibodies are the essential factors for several clinical manifestations associated with SLE. The primary importance of autoantibodies has been best demonstrated in experiments of B cell-depleted lupus mice. Lupus-prone mice depleted of B cells by a treatment with anti-IgM antibodies from birth completely failed to produce autoantibodies and generate glomerulonephritis (Cerny et al. 1987). However, it has never been clear whether all autoantibodies generated during the course of autoimmune diseases are indeed pathogenic. In fact, because of the occasional lack of correlation between serum levels of autoantibodies and clinical manifestations, it has long been suggested that the qualitative aspects of autoantibodies may be important for the pathogenesis of autoantibody-mediated cellular and tissue injuries. These include autoantibody's fine specificity, affinity, electrostatic charge, capacity to activate complement and capacity to fix Fcγ or complement receptors. In this respect, it is conceivable that the immunoglobulin class switching may result in a remarkable change of the pathogenic potential of autoantibodies, since this process can be accompanied with changes in antibody affinities, electrostatic charges and effector functions. However, until recently, this question could not be directly studied, because of the lack of suitable experimental models which easily reproduce some of the main pathological features usually observed in autoimmune diseases. Using monoclonal anti-DNA autoantibodies, attempts have been made to assess their pathogenic activities. However, no anti-DNA monoclonal antibodies (mAb) exhibited clear-cut nephritogenic activities. Although certain anti-DNA mAbs exhibit cross-reactivity to glomerular antigens (Faaber et al. 1986; Madaio et al. 1987), their pathogenic activities have never been demonstrated. In contrast, two types of monoclonal autoantibodies among lupus autoantibodies, i.e., anti-mouse red blood cell (MRBC) hemolytic antibodies and IgG3 anti-IgG2a rheumatoid factors (RF), have been recently shown to exhibit dramatic pathogenic activities. In this article, we will discuss molecular and cellular mechanisms responsible for the expression of pathogenic activities of these
two kinds of autoantibodies.

Pathogenicity of monoclonal anti-erythrocyte autoantibodies

NZB mice spontaneously develop autoimmune hemolytic anemia as a result of production of Coombs autoantibodies reacting with their own red blood cells (Howie and Helyer 1968). Although the molecular nature of these autoantigens responsible for the induction of autoimmune responses has not been well characterized, the fact that autoantibodies eluted from RBC of Coombs-positive NZB mice react only with MRBC but not with human, rat, sheep, guinea pig or rabbit RBC (Linder and Edgington 1972) has suggested the possible importance of the specificity of anti-MRBC antibodies for their pathogenic expression. In addition to their specificities, it is likely that the Ig heavy chain class of anti-MRBC autoantibodies plays a significant role in the pathogenesis of anemia by determining different effector functions, such as complement-dependent hemolysis, Fcγ or complement receptor-mediated phagocytosis, and multivalency-induced hemagglutination.

To study these questions, we have recently assessed the in vivo pathogenic activity of eight anti-MRBC mAbs, obtained from unmanipulated anemic NZB mice, by a single intraperitoneal injection of purified mAb, and determined their pathogenic mechanisms in relation to the specificities and effector functions of anti-MRBC mAb. Although all eight anti-MRBC mAbs react well with MRBC in vitro at 37°C as well as at 4°C, only five of them - two of four IgM mAb and three (two IgG1 and one IgG2a) of four IgG mAbs - are able to bind MRBC in vivo, causing anemia (Shibata et al. 1990a). It is significant that all the five pathogenic autoantibodies recognize only species-specific antigens (at least two distinct epitopes) on MRBC, while non-pathogenic antibodies recognize cross-reactive determinants present on RBC from many species such as human, rat, sheep, rabbit and chick (Table 1). In this regard, it should be mentioned that one IgM anti-MRBC mAb established by Caulfield et al. (1989) also reacts specifically with MRBC. These results illustrate the importance of the specificity for the

<table>
<thead>
<tr>
<th>mAb</th>
<th>Mouse</th>
<th>Rat</th>
<th>Sheep</th>
<th>Rabbit</th>
<th>Chick</th>
<th>Human</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1E10 (μ)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>4C8 (μ)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>31-9D (γ1)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>106-2H (γ1)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>34-3C (γ2a)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>34-2B (γ2b)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>103-7E (μ)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>106-10E (μ)</td>
<td>+</td>
<td>+</td>
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pathogenic activity of anti-MRBC autoantibodies. The importance of the anti-
MRBC specificity is further supported by the finding that among four IgM
anti-MRBC mAbs, only two pathogenic mAbs (1E10 and 4C8), whose specificities
differ from those of non-pathogenic ones, exhibit a marked hemagglutination
activity, which is responsible for the development of anemia. Although the
biochemical nature of antigenic determinants remains to be determined,
differences in the distribution and nature of target antigens could account for the
difference in the pathogenic activities of IgM anti-MRBC mAb.

Histological examinations have revealed that two different mechanisms are
responsible for the development of anemia induced by our anti-MRBC mAb
(Shibata et al. 1990a). In the case of IgG2a 34-3C and IgG1 105-2H anti-MRBC
mAbs, which bind efficiently to Fcγ receptors on phagocytes, the major cause of
anemia is apparently a rapid Fcγ receptor-mediated phagocytosis of opsonized
MRBC (Fig. 1A). This is confirmed by the fact that the development of anemia
due to these mAbs is completely prevented by the treatment with anti-Fcγ
receptor mAb (Shibata et al. 1990a), but markedly aggravated by the treatment
with granulocyte-macrophage colony-stimulating factor (GM-CSF) (Berney et al.
1992b), which promotes the proliferation and differentiation of the granulocyte
and monocyte/macrophage lineages and activates their phagocytic activities. In
contrast, two IgM (1E10 and 4C8) and IgG1 31-9D anti-MRBC mAbs, which fail
to mediate Fcγ receptor-dependent phagocytosis, cause anemia by marked seque-
stration of agglutinated RBC in spleens and livers (Fig. 1, B and C). Subsequent
liver damage and hemodynamic failure are likely to be additional contribution
factors to the animals' death. It should be mentioned that both 31-9D and 105-
2H mAbs are of the IgG1 class, yet a remarkable difference exists in their
pathogenic manifestations (Table 2). First, the 105-2H mAb requires almost 100
times more antibodies than the 31-9D mAb to induce significant anemia. Second,
in vitro, the 105-2H, but not 31-9D, mAb mediates Fcγ receptor-dependent
erythrophagocytosis. Finally, in vivo, the 105-2H mAb induces anemia as a
result of erythrophagocytosis, while the 31-9D mAb causes anemia due to the
sequestration of massively agglutinated RBC in spleens and livers. Since the
competitive binding assays have shown that these two IgG1 mAbs recognize
distinct epitopes, the different pathogenic activities of both IgG1 mAbs are likely
to be due to to differences in the distribution and nature of determinants recog-
nized by each mAb, further supporting the importance of the immunological
specificities of autoantibodies.

Although the specificity of autoantibodies is important, it is apparent that
other factors determine the pathogenic activity of anti-MRBC mAb. The best
example is the difference in the pathogenic potential between IgG2a 34-3C and
IgG1 105-2H mAb (Table 2). Although both mAbs appear to recognize the same
epitope, the competition experiment suggests that the affinity of 34-3C mAb to
MRBC is far higher than that of 105-2H mAb. In addition, in vitro experiments
Fig. 1. (A) Representative histological appearance of liver from BALB/c mice developing anemia after the injection of 2 mg of IgG1 105-2H mAb (HE; ×400). Note remarkable erythrophagocytosis by Kupffer cells. Similar lesions were observed in mice injected with IgG2a 34-3C mAb. (B and C) Representative histological appearance of liver from BALB/c mice developing anemia after the injection of 100 μg of IgG1 31-9D mAb. Note marked necrosis of hepatic parenchymal cells (B; HE: ×100) secondary to the accumulation of agglutinated RBC in hepatic sinusoids (C; HE: ×400). Identical histological changes were observed in mice developing anemia after the injection of two pathogenic IgM anti-MRBC mAb (1E10 and 4C8).
have shown that IgG2a 34–3C mAb exhibits greater binding to Fcγ receptor than does IgG1 105–2H mAb, owing to the known difference in the affinity of the Fc receptor for IgG2a and for IgG1/IgG2b. Both differences in the affinities of mAb for the target antigen and for the Fcγ receptor may well explain the fact that the pathogenic activity of IgG2a 34–3C mAb is approximately 10 times stronger than that of IgG1 105–2H mAb. In addition, our preliminary study has shown that an IgG1 switch variant of IgM 4C8 mAb fails to exhibit hemagglutinating activity, therefore unable to cause anemia. This supports the idea that, in the case of IgM 4C8 mAb, the heavy chain constant region plays a crucial role for its pathogenic activity, because of the multivalency-induced hemagglutinating property of the IgM class.

It is surprising to see that none of the anti-MRBC mAb, even of the IgM mAb, are able to lyse MRBC in vitro in the presence of complement. The development of anemia in C3-depleted and C5-deficient mice clearly indicates a minor role, if any, of complement-mediated hemolysis in the pathogenesis of anemia induced by our anti-MRBC mAbs (Shibata et al. 1990a). The failure of hemolysis by anti-MRBC mAb may be related to the particular structure of the target RBC antigens, since the structure and nature of the cell surface antigens appear to influence the capacity of antibodies to fix and/or activate complement (Bindon et al. 1988). Although one cannot exclude the possibility that combinations of antibodies against different epitopes might lead to complement-mediated hemolysis, it should be noted that NZB mice are congenitally deficient in hemolytic complement activity, yet develop severe autoimmune hemolytic anemia.

Although it is obvious that the anti-MRBC mAb studied may not represent the full range of pathogenic anti-MRBC autoantibodies occurring in NZB mice, the substantial variation of pathogenic activities observed among anti-MRBC could explain the remarkable individual variability in the progression of autoimmune hemolytic anemia in NZB mice. Since recent structural analysis of

### Table 2. Pathogenic activities of anti-MRBC mAb

<table>
<thead>
<tr>
<th>mAb</th>
<th>Anemia</th>
<th>Hemagglutinationa</th>
<th>FeR Bindingb</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1E10 (μ)</td>
<td>75 μg</td>
<td>+</td>
<td>–</td>
<td>Agglutination</td>
</tr>
<tr>
<td>4C8 (μ)</td>
<td>200 μg</td>
<td>+</td>
<td>–</td>
<td>Agglutination</td>
</tr>
<tr>
<td>103-7E (μ)</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>No</td>
</tr>
<tr>
<td>106-10E (μ)</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>No</td>
</tr>
<tr>
<td>31-9D (γ1)</td>
<td>15 μg</td>
<td>–</td>
<td>–</td>
<td>Agglutination</td>
</tr>
<tr>
<td>105-2H (γ1)</td>
<td>1,200 μg</td>
<td>–</td>
<td>+</td>
<td>Erythrophagocytosis</td>
</tr>
<tr>
<td>34–3C (γ2a)</td>
<td>100 μg</td>
<td>–</td>
<td>+++</td>
<td>Erythrophagocytosis</td>
</tr>
</tbody>
</table>

| aQuantity of mAb required for 50% decrease in hematocrit. |
| bIn vitro hemagglutinating and Fcγ receptor (FeR) binding activity. |
Autoantibodies has revealed the oligoclonal origin of autoimmune responses (Shlomchik et al. 1987), the selection of different autoreactive clones during the early course of autoimmune responses may markedly influence the onset and progression of autoimmune hemolytic anemia in NZB mice. In addition, it should be mentioned that we have recently observed remarkable differences in the therapeutic effect of various hematopoietic growth factors such as erythropoietin, GM-CSF and interleukin 3 on anti-MRBC mAb-induced anemia, depending on their pathogenic mechanisms (Shibata et al. 1990b; Berney et al. 1992b). Erythropoietin and interleukin 3 are effective on anemia caused by Fcγ receptor-mediated erythrophagocytosis and on the anemia by sequestration of agglutinated RBC, respectively, while GM-CSF aggravates the former form of anemia. We also observed considerable individual variations in the effects of various hematopoietic growth factors on the progression of anemia in NZB mice, which is likely to be a reflection of individual differences in the predominant mechanism of the anemia (Berney et al. 1992b). These findings may be of clinical importance for the elaboration of treatments of severe autoimmune hemolytic anemia.

Pathogenicity of IgG3 RF Cryoglobulins

Cryoglobulin activity of murine IgG3

MRL-lpr/lpr autoimmune mice spontaneously develop a lupus-like syndrome characterized by unique immunopathological manifestations such as arthritic lesions, necrotizing vascular lesions of the skin, ears and foot pads, and severe glomerulonephritis (Andrews et al. 1978). In parallel, they produce the most remarkable amounts of cryoglobulins among several SLE-prone mice. Although cryoglobulins from MRL-lpr/lpr mice are composed almost exclusively of IgG of polyclonal origin, the striking observation is that they are markedly enriched in IgG3, as compared with other IgG subclasses (Abdelmoula et al. 1989). This preferential precipitation of IgG3 in polyclonal cryoglobulins is not an exclusivity of autoimmune MRL-lpr/lpr mice, but can also be found in non-autoimmune mice after polyclonal stimulation of B cells with bacterial lipopolysaccharides or infection with malaria parasites (Abdelmoula et al. 1989).

Since IgG3 are selectively concentrated in spontaneous and induced cryoglobulins, the self-association and subsequent cryoprecipitation of murine IgG3 molecules are likely to be the principal mechanism responsible for the generation of cryoglobulins in mice. In fact, we and others have shown that the majority of IgG3 mAbs, from autoimmune and non-autoimmune mice, independently of their immunological specificities and origins, are capable of generating monoclonal cryoglobulins (Gyotoku et al. 1987; Spertini et al. 1989; Lemoine et al. 1992; Takahashi et al. 1993). The role of the γ3 heavy chain constant (Cγ3) region in the cryoglobulin generation has been most directly demonstrated by Ig class switch variant experiments, showing the association of the cryoglobulin activity with the IgG3 subclass (Depinho et al. 1986; Abdelmoula et al. 1989).
This unique property of murine IgG3 to self-associate is, however, not limited to cryoprecipitating mAb. The complete identity of the nucleotide sequences of the C\(_\gamma3\) regions of the non-cryoprecipitable J606 protein and a cryoprecipitable IgG3 6-19 mAb (Berney et al. 1992a) rules out an abnormality in the C\(_\gamma3\) region of either cryoprecipitable or non-cryoprecipitable IgG3 proteins. Apparently, an additional factor is required to form the cryoglobulin of IgG3 self-associating complexes. In this regard, it should be noted that the cryoprecipitation of anti-dinitrophenyl (DNP) IgG3 mAb is completely inhibited after the binding of monomeric anionic DNP-amino acid conjugates and can be enhanced by the binding of cationic conjugates (Spertini et al. 1989). This suggests that the electrostatic charges in the V regions may determine the cryoglobulin activity of IgG3 self-associating complexes. In view of remarkable pathogenic potential of IgG3 cryoglobulins, as discussed below, the introduction of new charged amino acid residues as a result of somatic mutations occurring during the course of autoimmune responses, as shown in the case of anti-DNA antibodies (Shlomchik et al. 1990; Tillman et al. 1992), may create more pathogenic cryogenerating autoantibodies.

**Induction of tissue lesions by IgG3 cryoglobulin monoclonal autoantibodies**

Using IgG3 monoclonal cryoglobulins, we have explored whether cryoprecipitable IgG3 mAbs are able to provoke tissue lesions in normal mice after intraperitoneal injection of IgG3-secreting hybridomas. One of the monoclonal IgG3 RF specific for IgG2a, clone 6-19, induces the most remarkable pathology (Gyotoku et al. 1987; Berney et al. 1992a). Five to 7 days after the injection of 6-19 IgG3 RF hybridoma into normal mice, a vascular purpura, which is the most common manifestation in patients with cryoglobulinemia (Brouet et al. 1974), develops in the skin of the ears, tails and foot pads, which are not well protected by hair from thermic variations. Histological examination of the skin lesions shows an intracapillary precipitation of cryoglobulins associated with an extensive infiltration of polymorphonuclear leukocytes around the vessels and into the subcutaneous tissue, and a massive extravasation of erythrocytes (Fig. 2). Moreover, the 6-19 mAb induces a severe acute glomerulonephritis (Fig. 3). At the initial phase, predominant glomerular lesions are characterized by exudation of polymorphonuclear leukocytes. Then, a progressive accumulation of cryoglobulins in the subendothelial spaces of glomerular capillary walls leads to the formation of glomerular lesions resembling the “wire-loop” lesion (Lemoine et al. 1992), characteristically described for lupus nephritis. In more advanced cases, a disseminated plugging of glomerular capillaries by voluminous cryoglobulins, often obstructing the capillary lumen, is noted.

Since the 6-19 mAb has both anti-IgG2a RF and cryoglobulin activities, it is important to determine the role of each activity in the pathogenicity of the 6-19 mAb. To answer this question, we have produced a 6-19-J558 hybrid antibody,
Fig. 2. Representative histological appearance of skin lesions after the intraperitoneal injection of 6-19 IgG3 RF hybridoma cells. Leukocytoclastic vasculitis is characterized by the infiltration of polymorphonuclear leukocytes and the extravasation of erythrocytes (HE; A: ×100 and B: ×200).
composed of 6–19 γ3 heavy chains and J558 λ1 light chains, which retains the cryoglobulin activity, but loses the anti-IgG2a RF activity (Reininger et al. 1990). Mice injected with this hybrid antibody develop glomerular lesions as severe as those induced by the 6–19 mAb, but completely fail to develop skin vasular lesions (Table 3). This strongly suggests that different pathological mechanisms
govern the development of each tissue lesion: both RF and cryoglobulin activities are required to induce cutaneous vascular lesions, while only the cryoglobulin activity is sufficient to cause glomerular lesions. This also indicates that the "wire-loop" lesion can be generated by the direct deposition of autoantibodies with a cryoglobulin activity, without the involvement of immune complex formation. These conclusions are further supported by the fact that the depletion of the corresponding autoantigen, IgG2a, in mice by treatment with anti-IgM antibodies from birth prevents the development of skin but not glomerular lesions after injection of 6-19 mAb (Reininger et al. 1990). Notably, an IgG1 class switch variant of IgG3 6-19 mAb, which loses the cryoglobulin activity, fails to generate both skin and glomerular lesions (Fulpius et al. 1993). Clearly, the cryoprecipitating activity of IgG3 RF mAb is a critical factor in the development of skin vasculitis and glomerulonephritis induced by IgG3 RF mAb. This is consistent with our recent finding that among a panel of anti-IgG2a RF mAb of different Ig isotypes, only the RF mAb able to cryoprecipitate - all from the IgG3 subclass - can induces skin and glomerular lesions (Table 4) (Berney et al. 1992a). The fact that the pathogenicity of autoantibodies is dramatically influenced by the Ig constant region emphasizes the importance of certain subpopulations of autoantibodies in the pathogenesis of autoantibody-mediated tissue lesions.

It should be mentioned that certain IgG3 cryoglobulin mAb cause only minimal glomerular changes (Gyotoku et al. 1987; Lemoine et al. 1992), although their serum levels of cryoglobulins are comparable or even higher than those of

<table>
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<tr>
<th>Table 3. Pathogenic activities of 6-19 mAb and its variants</th>
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<tbody>
<tr>
<td>mAb</td>
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<td></td>
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<tr>
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<td>6-19-J55811</td>
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<tr>
<th>Table 4. Pathogenic activity of anti-IgG2a RF mAb</th>
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<tr>
<td>---------------</td>
</tr>
<tr>
<td>IgM (7)*</td>
</tr>
<tr>
<td>IgG1 (3)</td>
</tr>
<tr>
<td>IgG2a (1)</td>
</tr>
<tr>
<td>IgG2b (5)</td>
</tr>
<tr>
<td>IgG3 (5)</td>
</tr>
<tr>
<td>IgA (1)</td>
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*Number of mAb studied.
mice injected with pathogenic IgG3 mAb. This would well explain the fact that
the quantity of circulating cryoglobulins does not always correlate with the degree
of nephropathy in patients with cryoglobulinemia (Verrout et al. 1982). Qualitative
features of cryoglobulins must be critical to determine the nephritogenic activity of cryoglobulins. The identification of the molecular substrate responsible for the nephritogenic activity of IgG3 cryoglobulin mAbs is of paramount importance in understanding molecular and cellular mechanisms responsible for the development of cryoglobulin-associated tissue lesions.

Protection of monoclonal IgG3 cryoglobulinemia by IgG3 non-cryogenerating monoclonal autoantibodies

Since both cryoprecipitating and non-cryoprecipitating IgG3 mAb are able to
interact with each other in a quantitatively similar manner, we have explored the
possibility that non-cryogenerating IgG3 mAb could inhibit the IgG3 cryoglobulin formation as a result of their nonspecific interaction, and thus inhibit the development of the tissue lesions by the IgG3 RF monoclonal cryoglobulin (Berney et al. 1991). In fact, excess amounts of non-cryogenerating IgG3 mAb, including 2-6D mAb, an anti-nuclear lupus autoantibody, are able to inhibit the cryoprecipitation of cryogenerating IgG3 mAb, and pretreatment with non-cryogenerating IgG3 mAb can prevent the development of tissue lesions induced by the 6–19 RF monoclonal cryoglobulin (Table 5). The lack of inhibitory effect by F(ab')2 fragments of non-cryoprecipitating mAb clearly indicates that the observed inhibition is not caused by a specific immunological interaction between cryogenerating and non-cryogenerating IgG3 mAbs. It is likely that because molecules of cryogenerating and non-cryogenerating IgG3 can associate with each other, either in heterologous or homologous aggregates, in a quantitatively similar manner, the physicochemical properties responsible for the cryoglobulin formation of these aggregates would tend to resemble those of the quantitatively dominant IgG3 mAb in the complex. Accordingly, excess of the non-cryogenerating compo-

<table>
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<th>Pretreatmenta</th>
<th>Serumb</th>
<th>Lesionsb</th>
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<tr>
<td></td>
<td>6-19Id</td>
<td>Cryoglobulin</td>
</tr>
<tr>
<td>2-6D</td>
<td>2.6±0.4</td>
<td>7±3</td>
</tr>
<tr>
<td>None</td>
<td>3.2±0.5</td>
<td>23±4</td>
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a20 mg of IgG3 non-cryoglobulin anti-nuclear monoclonal autoantibody (2-6D), derived from MRL-lpr/lpr mice, was injected intraperitoneally to BALB/c mice 8 hr before intraperitoneal injection of 7.5 mg of 6–19 mAb. Mice were sacrificed 24 hr after the 6–19 mAb injection.
bSerum levels of 6–19 idotype (Id; mg/ml) and cryoglobulins (µg/ml), and development of skin and glomerular lesions were determined at sacrifice.
nent could result in the inhibition of the cryoprecipitation of the cryogenerating one.

**Role of IgG3 cryoglobulins in autoimmune pathology of MRL-lpr/lpr mice**

The demonstration of the pathogenic activity of the cryoprecipitable monoclonal autoantibodies of the IgG3 subclass is significant, because cryoglobulins have long been suggested to be a potential source of tissue injuries in SLE, rheumatoid arthritis and related autoimmune diseases (Brouet et al. 1974). In addition to the results obtained with mAbs, a number of evidence supports the pathogenic importance of autoantibodies of the IgG3 subclass in murine SLE. First, recent studies on MRL-lpr/lpr x (MRL-lpr/lpr x C3H-lpr/lpr) backcross mice and on a new MRL-lpr/lpr substrain have shown a good correlation of IgG3 production with the development of lupus nephritis (Takahashi et al. 1991; Fossati et al. 1993). In addition, we have found a spontaneous production of IgG3 RF with cryoglobulin activity in a majority of sera from lupus-prone MRL-lpr/lpr mice, but rarely in C57BL/6- and C3H-lpr/lpr mice (Shibata et al. 1992). The lack of production of cryoprecipitable autoantibodies with immunopathological consequences may partly explain the development of only a limited autoimmune pathology in C57BL/6 and C3H mice bearing the lpr mutation (Izui et al. 1984). Second, the transfer of the xid (X chromosome-linked immune deficiency) gene, which causes a defect of IgM and IgG3 production, delays the development of lupus nephritis in three different lupus-prone mice including MRL-lpr/lpr mice (Steinberg et al. 1982; Smith et al. 1983; Steinberg et al. 1983). Finally, the role of cryoglobulins in the generation of joint lesions in MRL-lpr/lpr mice has been recently suggested (Itoh et al. 1991). However, as shown above, some IgG3 autoantibodies, lacking the cryoglobulin activity, could be protective against the development of IgG3 cryoglobulin-associated tissue lesions (Berney et al. 1991). Thus, the development of IgG3 cryoglobulin-mediated tissue lesions may be markedly influenced by the balance of formation of IgG3 autoantibodies with or without the cryoglobulin activity, i.e., pathogenic and protective IgG3 autoantibodies.

In view of the pathogenic importance of IgG3 cryoglobulins in murine autoimmune rheumatic diseases, it should be mentioned that human IgG3 have physicochemical properties similar to those of murine IgG3. All the human IgG3 myeloma proteins studied undergo a concentration- and temperature-dependent aggregation, though not always cryoprecipitation (Grey et al. 1968; Capra and Kunkel 1970). Although one should be aware of the fact that the qualitative aspect of cryoglobulins may be critically important for their expression of pathogenic activities, the presence of cryoprecipitable autoantibodies should be reassessed in relation to clinical manifestations to determine whether cryoprecipitable autoantibodies are useful and predictive markers of human rheumatic diseases.
Concluding remarks

Using two different kinds of monoclonal autoantibodies (anti-MRBC hemolytic antibodies and IgG3 RF cryoglobulins), we have demonstrated the importance of subpopulation(s) of autoantibodies in the pathogenesis of autoantibody-mediated cellular and tissue injuries. Studies on anti-MRBC mAbs have strongly suggested that their immunological specificity is a critical element for the expression of pathogenic activity. In addition, it has been well documented that the pathogenic potential can be markedly influenced by the Ig heavy chain constant regions, which determine Fcγ receptor-binding activity or multivalency-induced hemagglutinating activity. However, since the fine specificity and affinity to target antigens may not be identical among the mAbs used in the present study, further studies on Ig class switch variants are needed to reach a more definitive conclusion.

It is significant that the murine IgG3 isotype has the unique physicochemical property to self-associate and generate cryoglobulins. Strikingly, some of them induce severe glomerulonephritis and leukocytoclastic vasculitis, in which the cryoglobulin activity plays an essential role, while the RF autoantibody activity and subsequent immune complex formation play an additional role in the generation of leukocytoclastic vasculitis. However, it should be underlined that not all IgG3 cryoglobulins are nephritogenic, indicating the importance of qualitative features of IgG3 cryoglobulins for their pathogenic activity. Elucidation of the molecular basis responsible for the nephritogenic activity of IgG3 cryoglobulins is of paramount importance for the understanding of the pathogenesis of cryoglobulin-mediated tissue lesions. In addition, it is significant to note a positive correlation between the production of IgG3 cryoglobulins and the progression of glomerulonephritis in lupus-prone mice, particularly in mice bearing the lpr mutation. Since these data are still circumstantial, it is clear that one needs to undertake a more sophisticated experimental approach to address an important question: whether autoantibodies of the IgG3 isotype play a major role in the pathogenesis of murine SLE.

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