



GLYCOSYLATION IS INFLUENTIAL IN MURINE IgG3 SELF-ASSOCIATION

DAVID J. PANKA

Department of Microbiology, Boston University Medical School, 80 East Concord Street,
Boston, MA 02118, U.S.A.

(Received 3 March 1997; accepted 4 June 1997)

Abstract—In mice and humans, antibodies of the IgG3 isotype are unique in that they spontaneously self-associate. A consequence is the formation of cryoglobulins from some, but not all IgG3 molecules. Little is known about the structural basis of murine IgG3 self-association. A region of the CH3 domain that is unique to IgG3 antibodies is the presence of an extra glycosylation site at residues 471–473. It is known that glycosylation greatly influences solubility. It has also been shown by X-ray crystallography that glycosylated residues (specifically sialic acid) are influential in the contacts of the CH1 to CH2 as well as the CH2 to CH2 domains in a human IgG1 antibody. These findings provided evidence that a direct interaction exists between the glycosylated residues and other residues within the constant and/or variable domains. It was, therefore, important to determine whether the glycosylated residue in the CH3 domain of the IgG3 constant region is influential in self-association. We have found that removing the glycosylation site by site-directed mutagenesis of an IgG3 RF significantly reduced the self-associating ability of this antibody. © 1997 Elsevier Science Ltd.

Key words: glomerulonephritis, glycosylation, cryoglobulins

INTRODUCTION

In mice and humans, antibodies of the IgG3 isotype are distinguished by their capacity to spontaneously self-associate. This self-association has been proposed to be a product of Fc–Fc interactions (Spertini *et al.*, 1989; Abdelmoula *et al.*, 1989; Gytoku *et al.*, 1987). A consequence of this complex formation is a reduced low temperature dependent solubility of some, but not all IgG3 molecules resulting in the formation of cryoprecipitates. Cryoglobulins, in general, are associated with a number of autoimmune diseases in both humans and mice (Tzioufas *et al.*, 1988; Gripenberg *et al.*, 1988; Sikander *et al.*, 1989). IgG3 cryoglobulins are classified as Type I cryoglobulins, associated with immunoproliferative and autoimmune diseases such as SLE, Waldenstrom's macroglobulinemia, chronic lymphocytic leukemia, lymphocytic lymphomas, Sjogren's Syndrome, rheumatoid arthritis, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, essential cryoglobulinemia, multiple myeloma and cirrhosis of the liver (Brouet *et al.*, 1974; Meltzer and Franklin, 1966). Symptoms that have been associated with Type I cryoglobulins are vascular purpura, glomerulonephritis, including massive kidney deposits and renal failure, which quite often result in death. Older mice of the autoimmune strain MRL-lpr/lpr

(MRL/lpr) develop high titers of IgG3 antibodies, many of which are rheumatoid factors (RF). The resulting immune complexes contribute to a lupus-like autoimmune syndrome characterized by immunopathological manifestation such as necrotizing cutaneous vasculitis and glomerulonephritis (Murphy and Roth, 1978; Andrews *et al.*, 1978).

Although the structural basis of IgG3 cryoprecipitation has been partially defined, little is known about the structural basis of murine IgG3 self-association. All IgG3 antibodies studied to date have been shown to self-associate. It is also known that only IgG3 antibodies self-associate. An examination of identical V_H domains on IgG3 and non-IgG3 constant regions showed that only the IgG3 antibodies self-associated and that only the IgG3 RF showed any of the pathological consequences (glomerulonephritis, skin vasculitis) when injected into normal mice (Berney *et al.*, 1992). Furthermore, whereas antigen can influence cryoprecipitation (Spertini *et al.*, 1989; Panka *et al.*, 1995), antigen does not affect self-association. Finally, Fab and F(ab)₂ fragments of IgG3 antibodies do not self-associate nor inhibit the self-association of other IgG3 antibodies (Berney *et al.*, 1991; Chapman *et al.*, 1990). Based on these observations, it can be postulated that a portion of the structural correlates associated with IgG3 self-association and the resulting cryoprecipitation and pathological manifestations reside partially or fully in the CH2 and/or CH3 domains.

A region of the CH3 domain that is unique to IgG3 antibodies is the presence of an extra glycosylation site

Abbreviations: RF, rheumatoid factor, SCID, severe combined immunodeficient mice, V_H, Immunoglobulin heavy chain variable domain, V_L, Immunoglobulin light chain variable domain.

at residues 471–473 (asparagine–leucine–serine) (Wels *et al.*, 1984). It is known that glycosylation greatly influences solubility (Lawson *et al.*, 1983). It has also been shown by X-ray crystallography that glycosylated residues (specifically sialic acid) are influential in the contacts of the CH1 to CH2 as well as the CH2 to CH2 domains in a human IgG1 antibody (Middaugh and Litman, 1987; Silverton *et al.*, 1977). These findings provide evidence that a direct interaction exists between the glycosylated residues and other residues within the constant and/or variable domains. It was, therefore, important to determine whether the glycosylated residue in the CH3 domain of the IgG3 constant region is influential in self-association. In this report, it was found that removing the glycosylation site by site-directed mutagenesis of an IgG3 RF significantly reduced the self-associating ability of this antibody.

EXPERIMENTAL PROCEDURES

Hybridomas

The monoclonal MRL/lpr RFs 5-7B (IgG3,k; IgG2a^a specificity) (Jacobson *et al.*, 1994), and 6–12 (IgG3,k; IgG2a^a and IgG2a^b specificity; Shlomchik *et al.*, 1987), A/J anti-digoxin antibody 3E4 (IgG3,k; Panka *et al.*, 1995) and A/J anti-arsenate antibody 31–62 (IgG2a,k; Marshak-Rothstein *et al.*, 1980) were products of a fusion between splenocytes from their respective mice and sp2/0 (Shulman *et al.*, 1978).

Oligonucleotide-directed mutagenesis

A 2.3 kb SalI–SphI fragment of the IgG3 genomic constant region, cloned into M13 mp19, served as the template for the mutagenesis. The oligonucleotide, 5'(gagcgagacaggCtctctgtgtgtgg)3', (the mutated nucleotide is in bold and capitalized) containing the desired substitution was purchased from the Center for Human Genetics, Boston University Medical School. The mutant changing the asparagine at position 471 in the CH3 domain to serine was synthesized using the method of Nakamaye and Eckstein (1986), and a mutagenesis kit from Amersham (Arlington Heights, IL, U.S.A.). The mutation was verified by sequencing the entire 2.3 kb fragment, using the dideoxynucleotide sequencing method (Sanger *et al.*, 1977, 1980).

IgG3 Plasmid constructions, DNA transfections and analysis of transfectomas (Fig. 1)

The 2.3 kb SalI–SphI mutant N471S* fragment, cloned in M13 mp19 (intermediate E), was transferred to Bluescript SK (Sal–) containing the remainder of the IgG3 constant region (intermediate F). The SalI site had

previously been removed from the vector. The 5.5 kb HindIII fragment, containing the entire IgG3 constant region was inserted into the final expression vector (G), containing a V_H from a murine rheumatoid factor specific for IgG2a^a (5-7B; Jacobson *et al.*, 1994). Intermediates A, C and D were necessary to remove an extra HindIII site in this vector. The proper orientation of this vector was verified by digestion with EcoRI and StuI. This plasmid contains the selectable marker gpt, the *E. coli* xanthine–guanine phosphoribosyltransferase gene, which confers upon mammalian cells resistance to mycophenolic acid when xanthine is present in the medium (Mulligan and Berg, 1980). This plasmid was transfected into a myeloma cell line producing the germline equivalent of the 5-7B V_L chain (Jacobson *et al.*, 1994) by electroporation (Sambrook *et al.*, 1989). Transfectomas were selected in 96-well microtiter plates in DME supplemented with 20% fetal bovine serum, 50 µg/ml hypoxanthine, 6 µg/ml of mycophenolic acid, 250 µg/ml of xanthine and 5% Concavalin A supernatant. Transfectoma supernatants were initially screened for secreted IgG3 (as described). Positive transfectants were cloned by limiting dilution, injected into SCID for the production of ascites and purified and concentrated by ammonium sulfate precipitation. Additionally, an unmutated control vector was also transfected into the 5-7B V_L cell line.

IgG3 assay

IgG3 concentrations were determined by competitive RIA. Polystyrene plates were coated with goat anti-mouse IgG3 and blocked with 2 mg/ml BSA in PBS. IgG3 dilution (ascites, purified antibody, or culture supernatant) were incubated overnight with ¹²⁵I-3E4 (an A/J IgG3 anti-digoxin antibody), washed and counted. A standard preparation of 3E4 was used to determine accurate concentrations.

Measurement of rheumatoid factors (RF) by ELISA

Assay of serum samples or hybridoma supernatants for RF was performed as described previously (Wolfowicz *et al.*, 1988). Briefly, plates were first coated with NIP-BSA followed by culture supernatant of anti-NIP target antibody. The target was an MRL IgG2a, λ antibody. Dilutions of purified antibodies were added and incubated overnight at 4°C, washed and incubated overnight with alkaline phosphatase conjugated rat anti-mouse kappa chain. Plates were then washed and developed with p-nitrophenyl phosphate and the absorbance was read at 405 nm.

IgG3 self-association assay

A new self-association assay was developed, which is patterned after the rheumatoid factor assay from above. Plates were coated with anti-mouse λ1+λ2 (Pharmingen), followed by an IgG3, λ antibody (Pharmingen). Purified antibodies, containing the mutant IgG3 constant region and kappa light chain were added to the plates, incubated overnight at 4°C, washed and incubated over-

*Mutant antibodies are denoted by heavy chain position number, preceded by the wild type residue in the one letter amino acid code (IUPAC-IUB Commission on Biochemical Nomenclature, 1968).

The diagram illustrates the cloning strategy for IgG3 mutants. It shows the construction of several plasmids (A, C, D, E, F, G) and the steps involved in their creation and manipulation. Key features include the use of restriction enzymes (EcoRI, HindIII, SalI, SphI) and the resulting fragment sizes (4.5 kb, 11.2 kb, 5.5 kb, 2.3 kb). The strategy involves the insertion and subsequent mutagenesis of the IgG3 gene into a vector containing the 5-7VDJ region.

night with alkaline phosphatase conjugated rat anti-mouse kappa chain. Plates were then washed and developed with p-nitrophenyl phosphate and the absorbance was read at 405 nm. This assay system was quite reproducible but dependent upon relatively strong self-associating interactions (see Results). The assay was repeated in triplicate three times with two different wild-type transfectants (identical results) and one N471S transfectant. For each transfectant, three different purifications were assayed.

The mutant IgG3, k antibodies (1 μ g) were incubated with 15 ng (35 000 cpm) of ^{125} I-IgG3-k (3E4, an anti-digoxin antibody) overnight at 37°C. Electrophoresis of samples under non-denaturing and non-reducing (native) conditions was performed using 4% polyacrylamide slab gels. The gels were poured in 125 mM Tris, pH 6.8 buffer, with a running buffer for the gels of 25 mM Tris, 20 mM glycine, pH 8.8. The samples were diluted 1:1 with 125 mM Tris, pH 6.8 containing 1% low melting point agarose and immediately loaded into the wells of the gels.

RESULTS

The construction of the mutant antibody N471S was designed with the intention of ablating the unique glycosylation site in the C terminal portion of the CH3 domain of murine IgG3 antibodies. The mutant, along with the unmutated wild-type IgG3 constant region, was cloned into an expression vector which contained a V_H gene from a rheumatoid factor (5-7B) specific for IgG2a¹. These vectors were transfected into sp2/0 cells, previously transfected with the light chain from 5-7B (Jacobson *et al.*, 1994).

As shown in Fig. 2a, the N471S mutant had RF activity

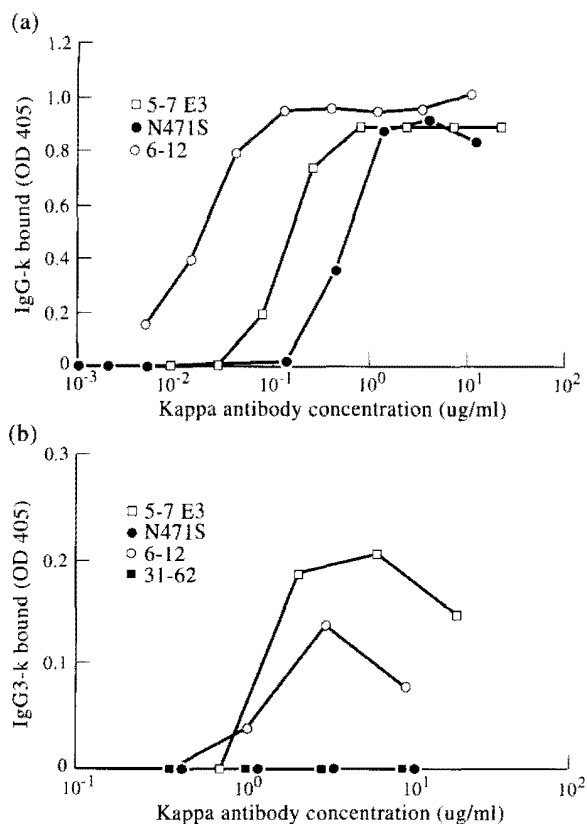


Fig. 2. Rheumatoid factor (a) and IgG3 self-association (b) assays of 5-7 E3 ($\square$), N471S ($\bullet$), 6-12 ($\circ$), an IgG3 RF and 31-62 ($\bullet$), an IgG2a anti-arsenate antibody. Results represent antibody bound in terms of OD at 405 nm.

nearly equivalent as the wild-type 5-7 E3 antibody (5-7 E3 is the cell line that resulted from transfection of the unmutated IgG3 constant region in conjunction with the 5-7B V_H gene into sp2/0, previously transfected with the light chain from 5-7B) implying that the heavy and light chains had combined properly and have retained the wild-type antibody combining site. However, whereas the wild-type antibody associated with the IgG3, λ antibody on the plate, the mutant N471S showed no affinity for the IgG3, λ on the plate (Fig. 2b). A non-IgG3 antibody (31-62) also did not have any affinity for the IgG3, λ , while a different IgG3 antibody (6-12) had affinity for the IgG3, λ , albeit slightly less than 5-7 E3. In order to show that the self-association is not an artifact of the assay, an iodinated IgG3 antibody (3E4, an A/J anti digoxin) was incubated with all of the above antibodies overnight at 37°C. The mixes were run on a native acrylamide gel. It has been postulated that complex formation is by non-covalent interactions. Therefore, SDS was not included in these gels. The native gel (Fig. 3a) showed that wild-type 5-7 E3 antibody complexes the iodinated IgG3 to a greater extent than N471S. Densitometry of the complex bands shows that the mutant N471S complexes the iodinated IgG3 about a third as effectively as the wild-type (Fig. 3b). Non-reducing SDS acrylamide gels of both the wild-type and mutants showed that greater than 90% of the expressed protein exists as intact antibody, consistent with the RF data and indicative that the heavy and light chains have combined and folded

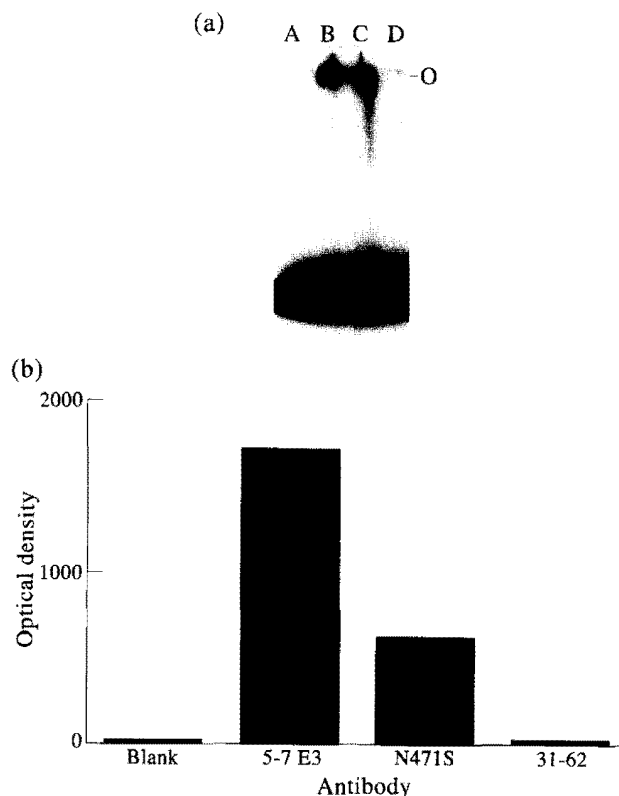


Fig. 3. (a) Native gel electrophoresis of complexes of IgG3 antibodies [(a) no antibody, (b) wild-type 5-7 E3, (C) N471S, (D) 31-62, an IgG2a antibody] and an ¹²⁵I-IgG3k antibody. The origin is denoted (O). The gel was run until the dye front ran off. (b) High molecular weight band was scanned and quantitated using a Molecular Dynamics Personal Densitometer and ImageQuant software.

properly, eliminating the possibility that the loss in association activity was due to improper heavy and light chain recombination (data not shown).

Although both assay systems measure the loss of self-association in mutant N471S, the extent of this loss is different. Using the ELISA system, we could measure greater than 30-fold loss in self-association. On the other hand, the gel system showed only a three-fold loss in self-association. An examination of the design of the assay systems sheds some light on this discrepancy. A comparison of Fig. 2a and 2b show that the relative 50% inhibition for the rheumatoid factor versus self-association assay for the wild-type 5-7 E3 is about 10-fold. The parent antibody 5-7B has been shown previously to have an affinity for IgG2a λ of $3.7 \times 10^5 \text{ M}^{-1}$ (Jacobson *et al.*, 1994). Therefore, an affinity of $3.7 \times 10^4 \text{ M}^{-1}$ for the binding of 5-7 E3 to the IgG3 λ antibody on the microtiter plate could be estimated. If we assume a third lower self-association for the mutant, an affinity of approximately 10^4 M^{-1} results. This is an especially low affinity constant. It may be that the binding of the mutant to the target is too weak to overcome the wash steps in the assay and as a result is lost before the addition of the alkaline phosphatase substrate. In the gel system, there are no washing steps. The complex is loaded directly onto the native acrylamide gel. This system is probably better suited for examining lower affinity self-associating IgG3

antibodies. Despite this difference, in both assays the mutant antibody had a reduced binding for the target antibody relative to the wild-type antibody.

DISCUSSION

This finding is similar to other studies involved with the role of glycosylation in both protein aggregation and cryoprecipitation. Studies on human antibodies have shown that glycosylation directly affects association. In two studies involving IgM Waldenstrom Macroglobulins with cryoglobulin activity, removal of carbohydrate residues by treatment with neuraminidase significantly reduced the cryoglobulin activity (Weber and Clem, 1981; Tsai *et al.*, 1977). In the latter report, cryoprecipitation of this protein was proposed to result from intermolecular binding of sialic acid residues. In an example of an unusual glycosylation event, an IgG monoclonal cryoglobulin had an extra glycosylation site in the amino terminal portion of the variable region of the heavy chain (Middaugh and Litman, 1987). As with the other proteins above, removal of most of the glycosylated residues by treatment with neuraminidase and a mixture of other glycosidases reduced the cryoglobulin activity. More direct evidence concerning the role of glycosylated residues in IgG aggregation comes from the X-ray diffraction studies of a complete human IgG1 cryoglobulin (Silverton *et al.*, 1977). The structure showed that carbohydrate residues have a very important role at the interface of the two CH2 and at the interface between CH2 and CH1. Finally, based solely on thermodynamic parameters, it has been proposed that the atypical behavior of monoclonal cryoglobulins can be explained by the formation of a few additional electrostatic contacts relative to normal immunoglobulins (Middaugh *et al.*, 1980). These increased polar contacts are proposed to be the determining factor in many of the cryoglobulins.

It is, therefore, not surprising that the extra glycosylation site of murine IgG3 antibodies has a significant role in its self-association and subsequent cryoprecipitation, resulting in the observed glomerulonephritis and skin vasculitis. It has already been reported that self-association, cryoprecipitation and disease pathology is not observed in non-IgG3 RFs. Therefore, we did not look at the effects of this non-self-associating mutant with respect to disease pathology. One could predict that the pathology would be the same as other non-IgG3 RF antibodies. These findings provide evidence that a direct interaction exists between the glycosylated residues and other residues within the constant and/or variable domains. Future plans will involve introducing the glycosylation site into the same position of an IgG2b and IgG1 antibody to see if self-association could be induced. If successful, it might be possible to create higher avidity antibodies as a consequence of self-association which can be used for therapeutic purposes.

Acknowledgements—This work was supported by an Arthritis Foundation Investigator grant.

REFERENCES

- Abdelmoula M., Spertini F., Shibata T., Gytoku Y., Luzuy S., Lambert P. H. and Izui S. (1989) IgG3 is the major source of cryoglobulins in mice. *Journal of Immunology* **143**, 526–532.
- Andrews B. S., Eisenberg R. A., Theofilopoulos A. N., Izui S., Wilson C. B., McConahey P. J., Murphy E. D., Roths J. B. and Dixon F. J. (1978) Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *Journal of Experimental Medicine* **148**, 1198–1215.
- Berney T., Shibata T. and Izui S. (1991) Murine cryoglobulinemia: Pathogenic and protective IgG3 self-associating antibodies. *Journal of Immunology* **147**, 3331–3335.
- Berney T., Fulpius T., Shibata T., Reininger L., Van Snick J., Shan H., Weigert M., Marshak-Rothstein A. and Izui S. (1992) Selective pathogenicity of murine rheumatoid factors of the cryoprecipitable IgG3 subclass. *International Immunology* **4**, 93–99.
- Brouet J.-C., Clauvel J.-P., Danon F., Klein M. and Seligmann M. (1974) Biologic and clinical significance of cryoglobulins. A report of 86 cases. *American Journal of Medicine* **57**, 775–788.
- Chapman P. B., Yuasa H. and Houghton A. N. (1990) Homophilic binding of mouse monoclonal antibodies against G_{D3} ganglioside. *Journal of Immunology* **145**, 891–898.
- Gripenberg M., Teppo A. M., Kurri P., Gripenberg G. and Helve T. (1988) Autoantibody activity of cryoglobulins and sera in systemic lupus erythematosus. Association of IgM class rheumatoid factors with Raynaud's syndrome. *Scandinavian Journal of Rheumatology* **17**, 249–254.
- Gytoku Y., Abdelmoula M., Spertini F., Izui S. and Lambert P. H. (1987) Cryoglobulinemia induced by monoclonal immunoglobulin G rheumatoid factors derived from autoimmune MRL/MpJ-lpr/lpr mice. *Journal of Immunology* **138**, 3785–3792.
- IAPAC-IUB Commission on Biochemical Nomenclature A. (1968) A one letter notation for amino acid sequences: tentative rules. *Journal of Biological Chemistry* **243**, 3557.
- Jacobson B. A., Sharon J., Shan H., Shlomchik M., Weigert M. G., Marshak-Rothstein A. (1994) An isotype switched and somatically mutated rheumatoid factor clone isolated from a MRL-lpr/lpr mouse exhibits limited intracloal affinity maturation. *Journal of Immunology* **152**, 4489–4499.
- Lawson E. Q., Hedlund B. E., Ericson M. E., Mood D. A., Litman G. W. and Middaugh C. R. (1983) Effect of carbohydrate on protein solubility. *Archives of Biochemistry and Biophysics* **220**, 572–575.
- Marshak-Rothstein A., Siekevitz M., Margolies M. N., Mudgett-Hunter M. and Geffer M. L. (1980) Hybridoma proteins expressing the predominant idiotype of the anti-azophenylarsonate response of A/J mice. *PNAS* **77**, 1120–1124.
- Meltzer M. and Franklin E. C. (1966) Cryoglobulinemia. A study of twenty nine patients. I. IgG and IgM cryoglobulins and factors affecting cryoprecipitability. *American Journal of Medicine* **40**, 828–836.
- Middaugh C. R., Lawson E. Q., Litman G. W., Tisel W. A., Mood D. A. and Rosenberg A. (1980) Thermodynamic basis for the abnormal solubility of monoclonal cryoimmunoglobulins. *Journal of Biological Chemistry* **255**, 6532–6534.
- Middaugh C. R. and Litman G. W. (1987) Atypical glycosylation of an IgG monoclonal cryoimmunoglobulin. *Journal of Biological Chemistry* **262**, 3671–3673.

- Mulligan R. C. and Berg P. (1980) Expression of a bacterial gene in mammalian cells. *Science* **209**, 1422–1427.
- Murphy, E. D. and Roth, J. B. (1978) Autoimmunity and lymphoproliferation: induction by mutant gene *lpr* and acceleration by a male-associated factor in strain BXSB mice. In *Genetic Control of Autoimmune Disease*. (Rose N. R., Bigazzi P. E. and Warner N. L., Eds), p. 107. Elsevier/North Holland, New York.
- Nakamaye K. and Eckstein F. (1986) Inhibition of restriction endonuclease *NciI* cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis. *Nucleic Acid Research* **14**, 9679–9698.
- Panka D. J., Salant D. J., Jacobson B. A., Minto A. W., Marshak-Rothstein A. (1995) The effect of VH residues 6 and 23 on IgG3 cryoprecipitation and glomerular deposition. *European Journal of Immunology* **25**, 279–284.
- Sambrook J., Fritsch E.F. and Maniatis T. (1989) *Molecular Cloning: A Laboratory Manual*. pp. 16.54–16.55. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Sanger F., Nicklen S., Coulson A. R. (1977) DNA sequencing with chain-terminating inhibitors. *PNAS* **74**, 5463–5467.
- Sanger F., Coulson A. R., Barrel B. G., Smith A. G. H. and Rol B. A. (1980) Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *Journal of Molecular Biology* **143**, 161–178.
- Shulman M., Wilde C. D. and Kohler G. (1978) A better cell line for making hybridomas secreting specific antibodies. *Nature* **276**, 269–270.
- Sikander F., Salgaonkar D. S. and Joshi V. R. (1989) Cryoglobulin studies in systemic lupus erythematosus. *Journal of Postgraduate Medicine* **35**, 139–143.
- Silverton E. W., Navia M. A. and Davies D. R. (1977) Three-dimensional structure of an intact human immunoglobulin. *PNAS* **74**, 5140–5144.
- Spertini F., Coulie P. G., Van Snick J., Davidson E., Lambert P. H. and Izui S. (1989) Inhibition of cryoprecipitation of murine IgG3 antidinitrophenyl (DNP) monoclonal antibodies by anionic DNP–amino conjugates. *European Journal of Immunology* **19**, 273–278.
- Tsai C.-M., Zopf D. A., Yu R. K., Wistar R. and Ginsburg V. (1977) A Waldenstrom macroglobulin that is both a cold agglutinin and a cryoglobulin because it binds N-acetylneuraminosyl residues. *PNAS* **74**, 4591–4594.
- Tzioufas A. G., Papadopoulos N. M. and Moutsopoulos N. M. (1988) Monoclonal cryoglobulins in primary Sjogren's syndrome. *Terapevticheskii Arkiv* **60**, 25–27.
- Wels J. A., Word C. J., Rimm D., Der-Balan G. P., Martinez H. M., Tucker P. W. and Blattner F. R. (1984) Structural analysis of the murine IgG3 constant region gene. *EMBO Journal* **3**, 2041–2046.
- Weber R. J. and Clem L. W. (1981) The molecular mechanism of cryoprecipitation and cold agglutination of an IgM1 Waldenstrom Macroglobulin with anti Gd specificity: sedimentation analysis and localization of interacting sites. *Journal of Immunology* **127**, 300–305.
- Wolfowicz C. B., Sakorafas P., Rothstein T. L., Marshak-Rothstein A. (1988) Oligoclonality of rheumatoid factors arising spontaneously in *lpr/lpr* mice. *Clinical Immunology and Immunopathology* **46**, 382–395.