EXPRESSION OF A PUBLIC IDIOTYPE BY HUMAN MONOCLONAL IgM DIRECTED TO MYELIN-ASSOCIATED GLYCOPROTEIN AND CHARACTERIZATION OF THE VARIABILITY SUBGROUP OF THEIR HEAVY AND LIGHT CHAINS

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Since the pioneering work of Kunkel et al. (1, 2), a number of studies established that human monoclonal IgM with a defined antibody activity such as rheumatoid factors (RF)[1] or cold agglutinins (CA) share crossreactive idiotopes (CRI).

In sharp contrast, it has been exceedingly difficult to obtain antiidiotypic reagents defining CRI on human monoclonal IgM reactive with myelin-associated glycoprotein (MAG). Whereas most studies yielded reagents that only defined private idiotopes on the immunizing IgM (3–6), we and others have previously shown that a restricted set of anti-MAG IgM shared CRI defined by a small subset of antibodies from rabbit antiidiotypic sera (7) or by mouse mAbs (8). The lack of broadly distributed CRI is not related to an heterogeneity of the fine specificity of these IgM since several studies indicate that most if not all react with a glucuronyl sulfated epitope carried by MAG and a peripheral nerve glycolipid, the sulfated 3-glucuronyl paragloboside (SGPG) (9, 10).

We report here the characterization, by a primate serum to human anti-MAG IgM, of combining site-related idiotope(s) shared by 9 of 10 IgM, and the assignment of light and heavy chains of 7 of these IgM to Ig variable subgroups as defined by their NH_2-terminal amino acid sequence.

Materials and Methods

Monoclonal IgM to MAG. Sera were obtained from 10 patients with monoclonal IgM and peripheral neuropathy. The specificity of the monoclonal IgM was assessed by immunoblotting analysis on human central nervous system myelin polypeptides. For some experiments,

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[1] *Abbreviations used in this paper:* CA, cold agglutinins, CRI, crossreactive idiotypes; MAG, myelin-associated glycoprotein; RF, rheumatoid factors; SGPG, sulfated 3-glucuronyl paragloboside.

MAG was prepared from delipidated myelin by lithium diodosalicylate and phenol extraction according to Quarles and Pasnak (11).

Partial NH_2-Terminal Sequence of IgM Heavy and Light Chains. Monoclonal IgM were isolated from sera by 40% saturated ammonium sulfate precipitation and were purified by gel chromatography followed by preparative zone electrophoresis. IgA, IgG, haptoglobin, and transferrin contaminants were removed on affinity adsorbents. Heavy and light chains were obtained by mild reduction performed with 10 mM 1-4 dithio-Dl-threitol and alkylation with 10% excess of [^{14}C]iodoacetic acid (7.5. 10^6 cpm/mol) (Amersham Corp., Amersham, UK); thereafter the chains were separated on a Sephadex G_{100} column equilibrated with 1 M acetic acid. The typing of variable regions from heavy and light chains was achieved by Edman sequence degradation, using 4N,N'-dimethylaminoazobenzene-4'isothiocyanate (Fluka, Buchs, Switzerland)/phenyl-isothiocyanate (Pierce Chemical Co., Rockford, IL) double-coupling method followed by thin-layer chromatography (12).

Antiidiotypic Serum. An adult marmoset was injected with purified IgM_{ROG}; the first immunization was performed subcutaneously in IFA (500 μg IgM in multiple sites) and booster injections were given every 15 d with IgM_{ROG} resuspended in saline buffer. Bleedings were obtained at monthy intervals and tested by double immunodiffusion against a panel of monoclonal anti-MAG IgM and normal serum. Bleedings that reacted strongly with anti-MAG IgM but not or weakly with normal serum were pooled and absorbed on Sepharose 4B beads coupled to normal human serum, polyclonal IgG, or four different monoclonal IgM devoid of anti-MAG specificity. This marmoset antiidiotypic serum was thereafter studied by ELISA.

ELISA Assays. The reactivity of marmoset antiidiotypic serum with anti-MAG or normal IgM was assessed by two ELISA assays. First, microtiter plates (Consortium de Matériel pour Laboratoire, Nemours, France) coated with 1 μg IgM/well for 18 h were blocked with 3% BSA in saline and incubated with serial dilutions of marmoset antiidiotypic serum; the binding of marmoset IgG was revealed with β-galactosidase-labeled goat antibodies to human IgG (Biosys, Compiègne, France) followed by substrate. No reactivity was recorded when the addition of marmoset antiidiotypic was omitted or when nonimmune marmoset serum was used. A similar assay was performed on plates coated with 10 μg/well of purified μ, κ, or λ chains.

Alternatively, monoclonal IgM were incubated for 12 h on plates precoated with 1 μg/well of mouse mAb to μ heavy chain. Thereafter, marmoset antiidiotypic antibody was added at a 10^{-1} dilution and the assay was developed with phosphatase alkaline–conjugated purified goat IgG to human (and primate) γ chains (Caltag Laboratory, San Francisco, CA) absorbed on mouse Ig, followed by substrate.

Inhibition experiments were performed as follows: anti-μ precoated plates were incubated with 5 μg/well IgM_{DEP} and revealed by marmoset antiidiotypic antibody (1/30 final dilution) previously incubated for 12 h with various amounts (10^{-2} to 10^2 μg) of anti-MAG or of an irrelevant monoclonal IgM followed by phosphatase alkaline–conjugated purified goat antibodies to human γ chains and substrate.

In other experiments, glutaraldehyde-pretreated plates were incubated with 10 μg of purified MAG, blocked with L-lysine and various amounts of anti-MAG IgM previously incubated for 18 h with a 1/50 final dilution of marmoset antiidiotypic serum were added. Plates were revealed with β-galactosidase–labeled goat antibodies to human μ chains followed by substrate (Biosys).

Results

Specificity of Marmoset Antiidiotypic Serum. As evidenced by immunoblotting experiments, the 10 IgM studied reacted with 90/100-kD polypeptides of myelin corresponding to MAG. In addition, IgM bound to purified MAG by immunoblotting or ELISA assays (not shown).

As shown in Fig. 1, 9 of 10 anti-MAG IgM reacted with marmoset antiidiotypic serum in two different assays. The immunizing IgM_{ROG} always yielded the highest optical densities, whereas IgM_{LOC} gave only background values similar to that ob-

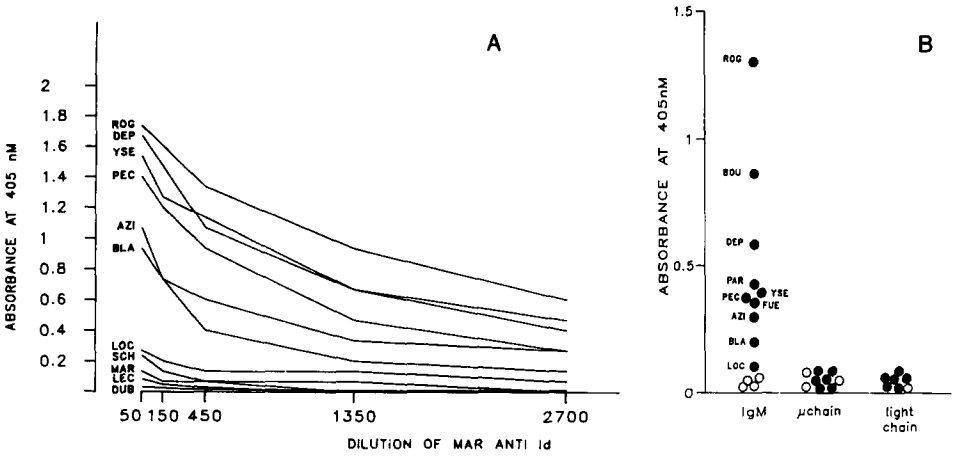

FIGURE 1. Reactivity of marmoset antiidiotypic serum with 10 anti-MAG IgM (*closed symbols*), irrelevant IgM (*open symbols*), or isolated heavy and light chains in ELISA. (*A*) Direct binding using serial dilutions of marmoset antiidiotypic serum. (*B*) IgM were captured on anti-μ-coated plates before addition of a 10^{-1} dilution of marmoset antiidiotypic serum (See Methods).

served with irrelevant monoclonal IgM. Inhibition studies confirmed the existence of crossidiotopes between IgM (Fig. 2 *A*) since the fixation of marmoset antiidiotypic serum to IgM_{DEP} (i.e., an IgM different from that used for immunization) was completely inhibited in the presence of anti-MAG IgM studied but was unaffected by an irrelevant IgM_{LEC}.

At least some of the shared idiotopes appeared to be related to the combining site of the IgM since incubation of four IgM with marmoset antiidiotypic serum inhibited their binding to MAG by ELISA (Fig. 2 *B*). In similar experiments, rabbit antisera to anti-MAG IgM that recognized private or semipublic idiotopes were not inhibitory (7; and unpublished data).

Marmoset antiidiotypic serum did not react either with isolated heavy and light chains from the six anti-MAG IgM tested or with variable subgroup matched μ chains or purified light chains (Fig. 1 *B*). Finally, various marmoset bleedings were tested by fluorescence on nerve sections for the presence of anti-antiidiotypic (ab3) antibodies: none were found, even in late bleedings after the end of booster immunizations.

NH_2-Terminal Sequence of Heavy and Light Chains of Anti-MAG IgM. Of seven IgM studied, only one possessed λ chains (AZI) with a $V_{\lambda II}$ variable region. Three κ chains (DEP, FUE, and LOC) belonged to the $V_{\kappa IV}$ subgroup with characteristic amino acids at position 9 and 12 (13). Two κ chains (BOU and BLA) were assigned to the $V_{\kappa I}$ subgroup, although several mutations existed in comparison to prototype sequence (Table I) since they had the relatively invariant amino acids of $V_{\kappa I}$ subgroup at position 3 and 13 and none of the characteristic amino acids of the other subgroups. Finally, IgM PEC belonged to the $V_{\kappa II}$ subgroup as shown by its NH_2-terminal sequence and the finding of an invariant methionine at position 89. Heavy chain NH_2-terminal amino acid sequence allowed the assignment to the V_{HIII} subgroup of 6 μ chains whose 10 first residues were identical to that of the V_{HIII} Gal prototype sequence (13). IgM AZI belonged to the V_{HII} subgroup with a single

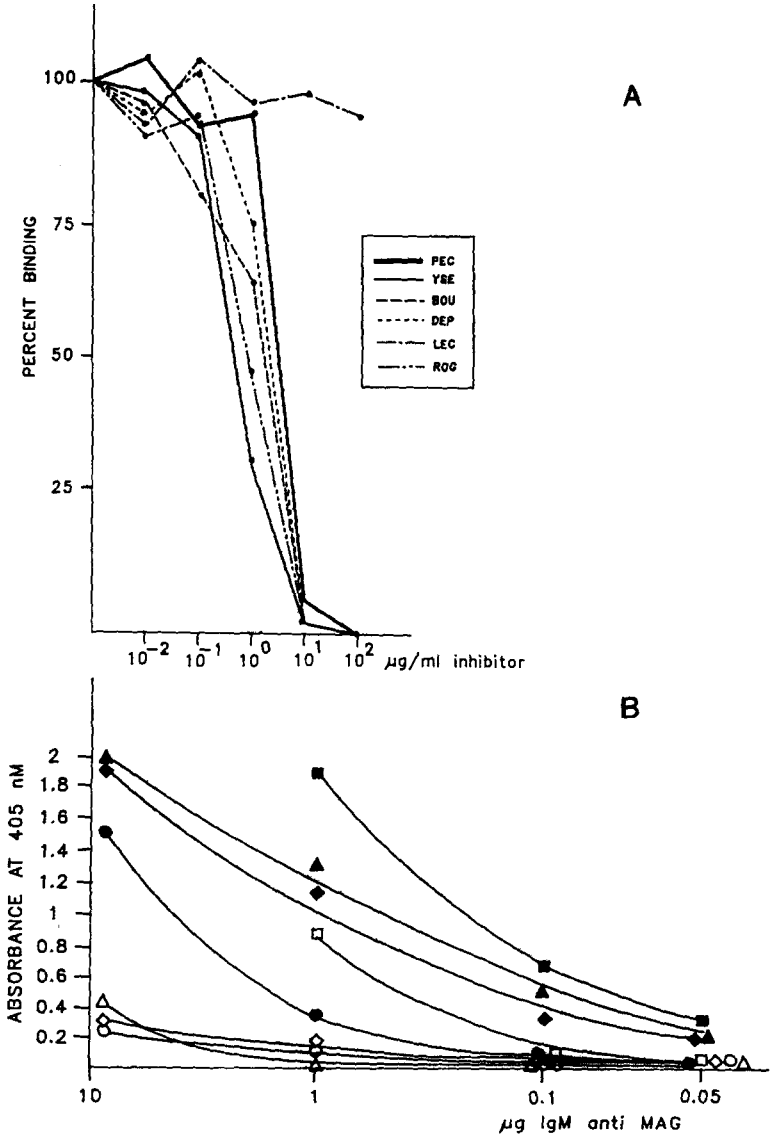

FIGURE 2. Shared idiotype(s) between anti-MAG IgM are combining site-related. (*A*) Anti-MAG $IgM_{ROG, DEP, YSE, BOU, PEC}$, but not irrelevant IgM_{LEC}, inhibit the binding of marmoset antiidiotypic serum to IgM_{DEP}-coated plates (See Materials and Methods). (*B*) Inhibition of binding of various concentrations of IgM_{ROG} (■), DEP (●), BOU (▲), PEC (t) to MAG-coated plates by a 1/50 dilution of marmoset antiidiotypic serum. Closed symbols represent optical density at 405 nM without serum and open symbols with marmoset antiidiotypic serum.

TABLE I

NH_2-Terminal Sequence of seven Anti-MAG IgM Light Chains Compared with that of Prototype Sequence of Variability Subgroups

	Sequence*												
Protein	1	2	3	4	5	6	7	8	9	10	11	12	13
$V_{\kappa I}$ SCW	D	I	Q	M	T	Q	S	P	S	S	L	S	A
IgM BOU	–	–	–	–	–	–	–	–	T	T	–	T	–
IgM BLA	–	–	–	–	–	–	–	–	T	T	–	–	–
$V_{\kappa II}$ TEW	D	I	V	M	T	Q	S	P	L	S	L	P	V
IgM PEC	–	V	–	–	–	–	–	–	V	T	–	–	–
$V_{\kappa IV}$ LEN	D	I	V	M	T	Q	S	P	N	S	L	A	V
IgM DEP	–	–	–	–	–	E	–	–	E	–	–	–	–
IgM FUE	–	–	–	–	–	E	–	–	–	–	–	–	–
IgM LOC	–	–	–	–	–	–	–	–	–	–	–	–	–
$V_{\lambda II}$ NEI	PCA	S	A	L	T	Q	P	A	S	V	S	G	S
IgM AZI	L	–	–	–	–	–	–	P	–	–	T	–	–

* Amino acids are designated by the single letter code. Only deviations from the prototype are shown.

amino acid substitution at position 7 (threonine for serine) when compared with the OU prototype sequence.

Discussion

Monoclonal IgM directed to MAG and nerve glycolipids recently attracted much interest since they may mediate the first instance of human autoimmune peripheral neuropathy. Although this matter remains controversial, intraneural injections of anti-MAG IgM as well as passive transfer experiments support this possibility (reviewed in reference 14). So far the antigenic and biochemical features of these IgM remain poorly characterized.

We show here that a primate antiserum to human anti-MAG IgM contains antibodies to a combining site-related public crossidiotype carried by 9 of 10 IgM tested. This antiserum was able to block the binding of monoclonal IgM to MAG and reacted with several IgM having κ or λ chains and μ heavy chain belonging to V_{HIII} (all IgM κ) or V_{HII} (one IgM λ) subgroups. In addition, inhibition studies suggested that all reactive IgM share a similar set of idiotopes. This marmoset antiidiotypic serum most likely recognizes conformational epitope(s) since it was unreactive with isolated light and heavy chains. These results contrast with those from previous reports where only private or highly restricted idiotypes were disclosed by rabbit polyclonal antisera and mouse mAbs antibodies (3–8; and unpublished data). The reasons for this discrepancy are not readily apparent. Of note, mouse and rabbit MAG are unreactive with human anti-MAG IgM and possibly the repertoire of these animals is submitted to idiotypic regulation depending on the structure of self antigens. Such species-restricted ab2 response have already been observed for RF (15). On the other hand, structural data (see below) showed that the combining site of these IgM is most often composed of V_{HIII}-related heavy chains paired with κ chains belonging to different subgroups of variability. Therefore, epitopes exposed at the surface of the IgM and that are immunogeneic in these species may be different from one IgM

to the other. In either instance, more related species, such as nonhuman primates, could more easily recognize human anti-MAG IgM combining sites. That such broadly crossreactive antiidiotypic antibodies are mostly directed against epitope(s) involved in (or close to) the combining site is not unexpected in view of the highly restricted specificity of anti-MAG IgM for a glucuronyl sulfated epitope of the carbohydrate moiety of MAG and peripheral nerve glycolipid SGPG (9, 10). However, slight differences in the fine specificity of anti-MAG IgM may exist (16, 17), and could account for the variable levels of binding to marmoset antiidiotypic serum of different anti-MAG IgM.

Dissection of the antigenic and structural relationship between individual monoclonal RF or CA in humans revealed that only a limited number of germline genes undergoing relatively few somatic mutations contribute to the generation of these monoclonal antibodies (18–20). Strikingly, a majority of monoclonal RF or CA possess light chains belonging to the $V_{\kappa III}$ subgroup. Most of them derive from a single (Hum 325) or closely related variable κ genes. Since other autoantibodies use also $V_{\kappa IIIb}$-related genes (21), a model for autoantibody generation has been proposed where the binding specificity is defined by the variable segment of heavy chains paired with multipotential light chains (18). Obviously anti-MAG IgM do not fit within this scheme; light chains from these IgM indeed belong to different V_κ variability subgroups ($V_{\kappa I}$, $V_{\kappa II}$, $V_{\kappa IV}$) and none are related to the $V_{\kappa III}$ subgroup so far. Interestingly half of the κ chains studied belong to the rare $V_{\kappa IV}$ subgroup, which is associated with <5% of sequenced light chains and coded by a single germline $V_{\kappa IV}$ gene.

These various light chains of anti-MAG were associated in all but one instance to V_{HIII}-derived μ heavy chains. Such a restriction of heavy chain variable regions is consistent with previous data for both RF and CA. However, at least three different V_{HI} variable genes are used by the RF belonging to the Wa CRI group and limited structural studies showed that salient features were the use of a J_{H4} region and of D_H regions of uniform size (22). Therefore, more structural data are needed to attain meaningful conclusions.

Altogether the present results indicate that anti-MAG IgM exhibit distinct antigenic and structural features as compared with those of other monoclonal IgM with a defined antibody activity. Whether or not they correspond to different pathogenetic processes (antigen-driven autoantibodies versus spontaneous production of autoantibodies from germline genes with few somatic mutations) presently remains speculative.

Summary

Most studies using rabbit or mouse antisera failed to detect CRI between human IgM directed to MAG. We show here that 9 of 10 such IgM express a public CRI as defined by a nonhuman primate antiserum. Shared idiotype is likely involved in (or close to) the combining site of those IgM since antiidiotypic serum inhibited the binding of IgM to MAG and reacted with IgM having different variable regions of light and heavy chains. Partial aminoterminal sequence of heavy and light chains showed that anti-MAG IgM use either λ chains (one IgM) or κ light chains (six IgM) of different variability subgroups ($V_{\kappa IV}$ in three instances, $V_{\kappa I}$ in two, and $V_{\kappa II}$ in one), whereas heavy chains belong to the V_{HIII} (six IgM) or to the V_{HII} (1 IgM)

subgroup. These features distinguish these IgM from other human monoclonal IgM with a defined antibody activity, such as rheumatoid factors or cold agglutinins.

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References

1. Williams, R. C. Jr., H. G. Kunkel, and J. D. Capra. 1968. Antigenic specificities related to the cold agglutinin activity of gamma M globulins. *Science (Wash. DC).* 161:379.
2. Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra. 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti-gammaglobulin activity. *J. Exp. Med.* 137:331.
3. Kahn, S. N. 1985. Human monoclonal IgM autoantibodies with restricted antigenic specificity for myelin express unrelated idiotypes. *J. Neurol. Sci.* 69:161.
4. Saito, T., W. Sherman, and N. Latov. 1983. Specificity and idiotype of M-proteins that react with MAG in patients with neuropathy. *J. Immunol.* 130:2496.
5. Murray, N., N. Page, and A. J. Steck. 1986. The human anti-myelin associated with glycoprotein IgM system. *Ann. Neurol.* 19:473.
6. Wehmeier, U., H. Rilke, U. Patzold, I. Schedel, and H. Deicher. 1987. Cross reacting idiotype of κ-monoclonal immunoglobulins M in sera of patients with Waldenström's macroglobulinemia. *Immunobiology.* 176:144.
7. Schmitt, C., K. Dellagi, E. Mihaesco, and J. C. Brouet. 1987. Detection of cross-reactive determinants shared by human monoclonal IgM reacting with myelin-associated glycoprotein. *J. Immunol.* 138:1442.
8. Evans, S. W., N. P. Gregson, S. L. Leibowitz, and N. R. Ling. 1984. Investigation of the idiotypic determinants of IgM monoclonal proteins associated with neuropathy. *Clin. Exp. Immunol.* 57:621.
9. Ilyas, A. A., R. H. Quarles, T. D. MacIntosh, M. J. Dobersen, B. D. Trapp, M. C. Dalakas, and R. O. Brady. 1984. IgM in a human neuropathy related to paraproteinemia binds to a carbohydrate determinant in the myelin-associated glycoprotein and to a ganglioside. *Proc. Natl. Acad. Sci. USA.* 81:1225.
10. Shy, M. E., T. Vietorisz, E. Nobile-Orazio, and N. Latov. 1984. Specificity of human M-proteins that bind to myelin associated glycoprotein. Peptide mapping, deglycosylation, and competitive binding studies. *J. Immunol.* 133:2509.
11. Quarles, R. H., and C. F. Pasnak. 1977. A rapid procedure for selectively isolating the major glycoprotein from purified rat brain myelin. *Biochem. J.* 163:635.
12. Chang, Y. J., D. Brauer, and B. Wittmann-Liebold. 1978. Microsequence analysis of peptides and proteins using 4-NN dimethylamino azobenzine 4' isothiocyanate-phenyl-isothiocyanate double coupling methods. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 93:205.
13. Kabat, E. A., T. T. Wu, H. Bilopsky, M. Reid-Miller, H. Perry, and K. S. Gottesman. 1987. Sequences of Proteins of Immunological Interest. U. S. Department of Health and Human Servies, Washington, DC.
14. Steck, A. J., N. Murray, K. Dellagi, J. C. Brouet, and M. Seligmann. 1987. Peripheral neuropathy associated with monoclonal IgM autoantibody. *Ann. Neurol.* 22:764.
15. Fong, S., T. A. Gilbertson, P. P. Chen, J. G. Karras, J. H. Vaughan, and D. A. Carson. 1986. The common occurence of internal image type anti-idiotypic antibodies in rabbits

immunized with monoclonal and polyclonal human IgM rheumatoid factors. *Clin. Exp. Immunol.* 64:570.

16. Tucker, G. C., K. Dellagi, C. Schmitt, J. C. Brouet, and J. P. Thierry. 1987. Heterogeneity of human anti-MAG IgM as revealed by their reactivity on avian embryonic tissue. *Clin. Exp. Immunol.* 67:352.
17. Quarles, R. H., A. A. Ilyas, and H. J. Willison. 1986. Antibodies to glycolipids in demyelinating diseases of the human peripheral nervous system. *Chem. Phys. Lipids.* 42:235.
18. Silverman, G. J., F. Goni, J. Fernandez, P. P. Chen, B. Frangione, and D. A. Carson. 1988. Distinct patterns of heavy chain variable region subgroup use by human monoclonal autoantibodies of different specificity. *J. Exp. Med.* 168:2361.
19. Radoux, V., P. P. Chen, J. A. Sorge, and D. A. Carson. 1986. A conserved human germline V_κ gene directly encodes rheumatoid factor light chains. *J. Exp. Med.* 164:2119.
20. Chen, P. P., D. L. Robbins, F. R. Jirik, T. J. Kipps, and D. A. Carson. 1987. Isolation and characterizations of a light chain variable region gene for human rheumatoid factors. *J. Exp. Med.* 166:1900.
21. Agnello, V., F. Goni, J. L. Barnes, M. T. de la Vega, and B. Frangione. 1987. Human rheumatoid factor crossidiotypes. II. Primary structure-dependent crossreactive idiotype, PSL2-CRI, present on Wa monoclonal rheumatoid factors is present on Bla and other IgMκ monoclonal antibodies. *J. Exp. Med.* 165:263.
22. Newkirk, M. M., R. A. Mageed, R. Jefferis, P. P. Chen, and J. D. Capra. 1987. Complete amino acid sequences of variable regions of two human IgM rheumatoid factors, BOR and KAS of the Wa idiotypic family, reveal restricted use of heavy and light chain variable and joining region gene segments. *J. Exp. Med.* 166:550.