CHARACTERIZATION OF HUMAN RHEUMATOID FACTORS WITH SEVEN ANTIIDIOOTYPES INDUCED BY SYNTHETIC HYPERVARIABLE REGION PEPTIDES

By POJEN P. CHEN,∗ FERNANDO GÓN,† RICHARD A. HOUGHTEN,‡ SHERMAN FONG,* ROBERT GOLDFIEN,* JOHN H. VAUGHAN,* BLAS FRANGIONE,§ AND DENNIS A. CARSON∗

From the Departments of *Basic and Clinical Research, and §Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California 92037, and the Department of Medicine and Pathology, New York University Medical Center, New York 10016

Rheumatoid factor (RF) autoantibodies against autologous IgG are found in the sera of most patients with rheumatoid arthritis (RA) (1). IgM-RF, and particularly IgG-RF comprise a large part of the immune complexes that are abundant in rheumatoid synovial fluid, and may contribute to complement consumption and chronic tissue damage (2).

RF are not specific for RA. The autoantibodies have been detected in the sera of some normal individuals (3) and in the culture supernatants of polyclonally activated normal lymphocytes (4). IgM-RF autoantibodies are produced regularly during primary and secondary immune responses in humans and mice (5–7). In a well-defined murine system, IgM-RF synthesis was dependent upon both immune spleen cells and the relevant antibody (8, 9). These results strongly suggest that IgM-RF are a physiologic component of the immune system (7, 10).

Conceivably, antibodies with evolutionarily significant protective or regulatory functions may be encoded by germ line Vh and Vl (variable heavy and light chains) genes. Crossreactive idiotypes (CRI) represent serologic markers for Ig Vh and Vl sequences. In elegant studies, Kunkel and coworkers (11) described two CRI, (Wa and Po), on Waldenstrom's macroglobulins with IgM-RF activity. The mutually exclusive Wa and Po CRI were expressed, respectively, by 65% and 20% of monoclonal IgM-RF. Amino acid sequence analyses (12, 13) revealed that Wa-CRI+ IgM-RF have similar L chains, and that Po-CRI+ proteins have similar H chains.

To gain insight concerning structural and genetic relationships among a larger series of IgM-RF proteins in an outbred human population, we generated two

This work was supported in part by grants AM35218, AM25443, AM07144, AG04100, AM21175, NS18113, and RR00833, and by AM02594 (awarded to F. Goni and B. Frangione) from the National Institutes of Health, Bethesda, MD. P. Chen is the recipient of an Arthritis Investigator Award from the Arthritis Foundation. This is publication number 3853BCR from the Research Institute of Scripps Clinic.

†Abbreviations used in this paper: BBS, borate-buffered saline; BSA, bovine serum albumin; CDR, complementarity-determining region; CFA, complete Freund's adjuvant; CRI, crossreactive idiotype; D, diversity region of Ig; ELISA, enzyme-linked immunosorbent assay; H, heavy chain of Ig; J, joining region of Ig; KLH, keyhole limpet hemocyanin; L, light chain of Ig; RA, rheumatoid arthritis; RF, rheumatoid factor; V, variable region of Ig.
different antiidiotypic antibodies against short, primary sequence-dependent idiotypic determinants on RF H and L chains (14, 15). In initial experiments, an antiidiotypic antibody was induced by immunization with a synthetic peptide corresponding to the H chain third hypervariable region (complementarity determining region) (CDR) of the Wa-CRI+ IgM-RF Sie. The antipeptide antibody reacted with the Sie protein, but not with pooled human Ig. A subsequent antiidiotypic, produced by immunization with a synthetic peptide corresponding to the second CDR of the IgM-RF Sie L chain, recognized 10 out of 12 human IgM-RF paraproteins (15, 16). The latter results suggested that the majority of IgM-RF L chains are encoded by a single germline Vκ gene, or a family of closely related Vκ genes (16).

In the present experiments, the CDR structures on RF H and L chains have been probed with seven different peptide-induced antiidiotypic antibodies. A panel of 16 human monoclonal IgM-RF was screened by immunoblotting with the antipeptide antibodies. The results show that the majority of RF share at least two L chain-associated CRI, but not H chain-associated idiotypes. Apparently, antibodies with anti-IgG autoantibody activity repeatedly utilize a common κ L chain sequence, but diverse H chain sequences. Thus, in contrast to the L chains, RF H chains may employ a large number of Vκ genes.

Materials and Methods

Preparation of Synthetic Peptides and Antipeptide Antibodies. The seven synthetic peptides listed in Table I (17–19) were prepared by Merrifield’s (20) solid-phase method, slightly modified as previously described (14, 15, 20, 21). Each peptide (P) was assigned a four-letter code, designating the corresponding parental protein (S, W, P), heavy or light chain (H, L), and complementarity determining region (1–3). The peptides were conjugated via cysteine to keyhole limpet hemocyanin (KLH) with m-maleimidobenzoyl N-hydroxysuccinamide ester (22). In initial experiments, rabbits were immunized twice with conjugates emulsified in complete Freund’s adjuvant (CFA), then boosted twice with glutaraldehyde-

| Peptide name* | Protein | Residues spanned‡ | Amino acid sequence§ |
|---------------|---------|------------------|---------------------|
| PSL2          | Sie     | 49–61            | YGASSRATGIPDR(C)    |
| PSL3          | Sie     | 88–99            | CQQYGSSPQTFG        |
| PS1H2         | Sie     | 49–65            | GSPAKWTDPFGVYIKWE(GGC) |
| PS1H3         | Sie     | 95–102           | EWKGQVNVNFVDV(GGC)  |
| PWH2          | Wol     | 49–65            | GQIPLRFNGEVNGPVS(V(GGC) |
| PWH3          | Wol     | 95–102           | EYFGFDSYDDY(A(GGC)  |
| PPH2          | Pom     | 49–65            | AWKYENGNDHYADSVNG(GGC) |
| PPH3          | Pom     | 95–102           | DAPYVSPFFA(H(GGC)   |

* The first letter, P, designates the peptide; the second letter designates the corresponding parent protein; the third letter designates the heavy or light chain, and the fourth letter designates the CDR.
‡ Numbered according to Kabat et al. (17).
§ The amino acid sequences were reported previously (13, 18, 19). The underlined residues belong to the adjacent framework regions (FR). The residues within the parentheses were added to the C-terminus for coupling purposes. The one-letter amino acid code is used.
crosslinked peptides in incomplete adjuvant, as described previously (14, 15). Thereafter, antisera were obtained and characterized. Subsequently, the immunization procedure was changed to two injections of conjugates in CFA at 1-mo intervals. This scheme generated antibodies with the same specificity as the original protocol.

Preparation of Proteins. Plasma or purified proteins from patients with monoclonal IgM cryoglobulins were generously provided by Drs. J. D. Capra (University of Texas Southwestern Medical School, Dallas, TX) G. Abraham (University of Rochester School of Medicine, Rochester, NY) J. Johnson (Nashville, TN) D. Normansell (University of Virginia, Charlottesville, VA) and H. Metzger (National Institutes of Health, Bethesda, MD). The IgM cryoglobulins were purified by repeated precipitation at 4°C, followed by chromatography on Sephadex G-200 in 0.1 M acetic acid (23), or Ultrogel AcA 22 in 0.2 M sodium acetate, pH 3.5. Pooled human IgG was prepared from Cohn Fraction II (Sigma Chemical Co., St. Louis, MO) by DEAE-cellulose chromatography in 0.01 M sodium phosphate, pH 8.0. Bence-Jones κ L chains, prepared from patients' urine by ammonium sulfate precipitation, were the generous gift of Dr. Elliott Osserman (Columbia University College of Physicians and Surgeons, New York). The κ subgroups of the various L chains were kindly determined by Dr. Alan Solomon (University of Tennessee, Knoxville, TN) using subgroup-specific antisera (24).

Enzyme-linked Immunosorbent Assay (ELISA). The ELISA method for idiotype detection has been described previously (14, 15). Briefly, antigens in borate-buffered saline (BBS), 0.1 M borate, 0.2 M NaCl, pH 8.2, were used to coat microtiter plates at 100 μl/well. After blocking with 1% bovine serum albumin (BSA), 100 μl of samples diluted in BBS containing 0.5% BSA were distributed to duplicate wells. The plates were incubated for 3 h at room temperature, and the bound antibodies were quantified by the binding of human IgG-adsorbed, alkaline phosphatase-labeled goat anti-rabbit IgG antibodies (Kirkegaard and Perry, Gaithersburg, MD).

Immunoblotting. The reactivities of antipeptide antibodies with Ig L and H chains were analyzed by the Western blot method (25), with some modifications (14, 16, 26). Briefly, 20 μg of each individual protein (13, 23) or pooled human IgG in gel buffer were loaded onto each slot of a 10% polyacrylamide slab gel containing 0.1% sodium dodecyl sulfate (27). After electrophoresis (2.5 h at 30 mA), the proteins in the gel were transferred electrophoretically to nitrocellulose papers (2 h at 70 V). Protein-binding sites on the papers were quenched with 0.01 M phosphate-buffered saline, pH 7.2, containing 5% BSA for 1 h at room temperature. Subsequently, the papers were overlaid with the indicated antisera in BBS for 1 h. After washing with BBS, the papers were incubated with 125I-labeled protein A (1 mCi/mg, 2 x 10^6 cpm/ml) for another hour. Then, papers were washed with BBS, dried, and finally exposed to Kodak XAR-5 film overnight at -70°C.

Results

Expression of Two Major CRI on Human IgM-RF L Chains. All rabbits produced high titers (1:10,000) of antipeptide antibodies, as measured by ELISA (data not shown). Depending upon the immunizing peptide, approximately half of the animals produced antibodies that reacted with the L or H chains of the parent protein, as assessed by immunoblotting. These latter antibodies were used to analyze idiotypic crossreactions among multiple IgM-RF.

Previously, we showed that 10 out of 12 (83%) human monoclonal IgM-RF bear the PSL2-associated CRI marker (16) (Table II). Subsequently, five additional IgM-RF were analyzed for the PSL2-CRI. Fig. 1a (left) shows that anti-PSL2 antibodies react with three out of these five new IgM-RF. Together with our previous data, 13 out of 17 (76%) IgM-RF express the PSL2-CRI. This figure is close to the 65% of IgM-RF that express the Wa-CRI (11, 28). The
results lend further support to the possibility that the second CDR on RF light chains (L2) is the structural correlate of the Wa-CRI.

It remains controversial whether somatic mutations in Ig variable regions are linked to the Ig class switch (29-31), or whether they occur commonly in IgM molecules (32-34). The IgM-RF L chains, which bear the PSL2-CRI, are likely encoded by a single Vk gene or a family of closely related Vk genes (16). To estimate the extent of diversification of PSL2+ RF L chains, antibodies were generated against the third CDR of the IgM-RF Se L chain (PSL3). Immunoblotting with the two antipeptide antibodies enabled us to determine whether the anti-PSL2- and anti-PSL3-defined sequences cosegregated among a panel of IgM-RF proteins from unrelated individuals.

Three rabbits were immunized with the PSL3 peptide conjugated to KLH. One rabbit antisera reacted well with isolated Se L chains and with the intact IgM-RF Se protein (Table III and Fig. 2). Antibody binding to both intact Se, and to Se L chains was inhibited by the free PSL3 peptide (data not shown).

The reactivity of the anti-PSL3 antibody against a panel of 16 human monoclonal IgM-RF was analyzed by immunoblotting. As shown in Fig. 2, the anti-PSL3 bound to only two out of three known Wa-CRI+ RF, indicating that the third CDR of the L chain is unlikely to be the structural basis of the Wa-CRI. Fig. 1b (right) and Fig. 3 show that anti-PSL3 reacts with three additional RF, but with none of six λ Bence-Jones proteins. Among these six L chains, Eps and

### Table II

| Idiotype Expression of 17 Human Monoclonal IgM-RF |
|-----------------------------------------------|
| IgM-RF | PSL2* | PSL3* | PSH3 | PWH2 | PWH3 | PPH2 | PPH3 |
|--------|-------|-------|------|------|------|------|------|
| Cur    | ++    | ++    | -    | -    | -    | -    | -    |
| Gar    | ++    | ++    | -    | -    | -    | -    | -    |
| Glo    | ++    | ++    | -    | -    | -    | -    | -    |
| Got    | ++    | ++    | -    | -    | -    | -    | -    |
| Neu    | ++    | +     | -    | -    | -    | -    | -    |
| Pal    | ++    | ++    | -    | ND   | ND   | ND   | ND   |
| Pay    | ++    | ++    | -    | -    | -    | -    | -    |
| Pom    | -     | -     | -    | -    | ++   | ++   | ++   |
| Sie    | ++    | ++    | ++   | -    | -    | -    | -    |
| Wol    | ++    | -     | ++   | ++   | -    | -    | -    |
| Boc    | ++    | +     | -    | -    | -    | -    | -    |
| Flo    | ++    | ++    | -    | -    | -    | -    | -    |
| Gal    | -     | ++    | +    | -    | -    | -    | -    |
| Lew    | ++    | -     | -    | -    | -    | -    | -    |
| She    | -     | -     | -    | -    | -    | -    | -    |
| Lay    | ++    | ND    | -    | ND   | ND   | ND   | ND   |
| Teh    | ++    | ND    | -    | ND   | ND   | ND   | ND   |
| Total positive | 13 | 11 | 2 | 1 | 1 | 1 | 1 |
| Total assayed | 17 | 16 | 17 | 14 | 14 | 15 | 15 |
| Percent positive | 76 | 69 | 12 | 7 | 7 | 7 | 7 |

* The results of five RF are in Fig. 1a. The other results are from Chen et al. (16).

---

1. D. A. Carson and B. Frangione. 1985. Preferential Vk and Jk utilization in human monoclonal IgM autoantibodies (rheumatoid factors). Manuscript in preparation.

2. ND, not done.
Wie belong to the κ I subgroup, Mcg belongs to the κ II subgroup, and the rest belong to the κ III subgroup. Thus, the anti-PSL3 defines a primary sequence-dependent CRI that is expressed by 69% of IgM-RF paraproteins (Table II). Furthermore, the PSL2- and PSL3-defined CRI are often found together on the same L chain.

**Expression of Three H Chain Idiotypes Associated with Two Wa-CRI⁺ IgM**
IDIOTYPES OF HUMAN RHEUMATOID FACTOR

RF. Synthetic peptide–induced antibodies and immunoblotting were used similarly to study the idiotypes associated with RF H chains. Four peptides were synthesized, corresponding to the second and third H chain CDR of the IgM-RF Sie and Wol. These two proteins express the dominant Wa-CRI. Antisera from PSH2-immunized rabbits reacted with the free peptide, but not with Sie H chains. In contrast, anti-PSH3 from all four immunized rabbits reacted specifically with the intact IgM-RF Sie, and with isolated Sie H chains (14), Fig. 4 and Fig. 5c (lower left). 17 different IgM-RF were probed with the PSH3-induced antiidiotype. Only one RF, other than Sie, was weakly positive.

Both the PWH2 and PWH3 peptides induced rabbit antibodies that recognized the isolated H chains of IgM-RF Wol. Fig. 5d (lower right), and Figs. 6 and 7 show that only Wol is positive for the PWH2- and PWH3-defined idiotypes, among 14 IgM-RF analyzed.

Expression of Two H Chain Idiotypes Associated with Po-CRI RF. The Po-CRI antigen is present on 20% of human monoclonal IgM-RF (11). Po-CRI+ IgM-RF have similar \( V_\alpha \) sequences (13), particularly in the second and third CDR (H2 and H3). Peptides corresponding to the H2 and H3 regions of the IgM-RF Pom

| CRI: | Sie | Wa | Wa | Wol | Wa | Po | Po |
|------|-----|----|----|-----|----|----|----|
| IgM-RF: | Sie | Wol | Glo | Lay | Po | Pom | Pom |

Fig. 2. Immunoblot analysis of RF bearing either the Wa-CRI or the Po-CRI. The experiment was performed as described in Fig. 1, except that only 8 \( \mu \)g of Wol was loaded, and that polyspecific rabbit anti-human Ig antibodies (IgG fraction, at 5 \( \mu \)g/ml) were used to show the relative amount of polypeptides blotted onto nitrocellulose paper.
CHEN ET AL.

**FIGURE 3.** Immunoblot analysis of six Bence-Jones κ L chain proteins. The methods are the same as in Fig. 2. Protein Mcg is the abbreviation of McGee.

**FIGURE 4.** Immunoblot analysis of the anti-PSH3 antiserum. The methods are the same as in Fig. 1.
Figure 5. Immunoblot analysis of six IgM-RF with four different peptide-induced antidiotype antisera. The experiments were performed as in Fig. 1, except that the papers were reacted first with either anti-PPH2 (a) or anti-PPH3 (b), and then with either anti-PSH3 (c) or anti-PWH3 (d). This modification was necessitated by the very limited supply of these IgM-RF.
are synthesized (Table I) and used to immunize rabbits. All PPH3-immunized rabbits, and one of two PPH2-immunized rabbits, produced antibodies reactive with the parent protein Pom (Table III). Fig. 5 (a and b, top) and Fig. 8 show
that anti-PPH2 and anti-PPH3 antibodies reacted well with Pom H chains, but not with the other RF analyzed.

Discussion

Synthetic peptides were used to generate seven specific antiidiotypic antibodies against human IgM-RF. These reagents were tested against a large panel of IgM-RF paraproteins. The results demonstrate that the majority of human monoclonal IgM-RF share two primary sequence–dependent CRI, corresponding the second CDR and third CDR of the κ L chain. An analysis of the amino acid sequences of four RF expressing both the PSL2- and PSL3-CRI revealed complete identity in the whole Vκ gene region (35). In addition, five RF bearing the PSL2-CRI have almost identical sequences, with only one to four different amino acid residues per RF L chain (12).

In contrast to the recurrent CRI on IgM-RF L chains, the H chain idiotypes are extremely private. Thus, all of four antiidiotypes against synthetic peptides, corresponding to different H chain CDR, reacted only with the parent proteins. A fifth peptide-induced antiidiotype reacted weakly with one additional IgM-RF. These results suggest either that RF H chains are encoded by a number of different Vh and Dh (diversity regions of H chain of Ig) genes, or that IgM-RF H chain sequences reflect an unusually high degree of somatic mutation in a limited number of Vh genes, as well significant variation in VDJ (variable, diversity, joining region) gene joining.

Crossreactive idiotypes represent phenotypic markers for Vh and Vκ genes (36,
For L chains, the $V_L$ genes encode amino acid residues up to about position 95 (i.e. including 2/3 of CDR3 region) (38, 39). The $V_H$ genes encode amino acid residues up to about position 94, the last amino acid residue of the third framework region. The third CDR of H chains (H3) is encoded by $D_H$, $J_H$, and nucleotides of unknown origin (40-42). Only 5-10 $D_H$ genes, and five $J_H$ genes, have been identified in humans and mice. However, imprecise joining at the VD and DJ junctions together with the inclusion of other nucleotides, make the third H chain hypervariable region the most heterogeneous among the six Ig CDR (42-44). This conclusion was demonstrated elegantly by primary sequence analysis of eight murine IgM antigalactan antibodies (45, 46). Thus, it is not surprising that all three antibodies against synthetic peptides, corresponding to the third CDR of RF H chains, recognized private idiotypes.

We anticipated that antibodies against one or both synthetic peptides, representing the second CDR of IgM-RF H chains, would recognize a CRI determinant. In the IgM antigalactan system, the second CDR of the H chain was identical in seven out of eight cases (45). The eight H chains were all encoded by a single $V_H$ gene (34), and four had identical amino acid sequences through the whole variable region. Seven of the IgM antigalactan antibodies also had identical sequences through the whole $V_k$ region (46). By analogy, the fact that the monoclonal IgM-RF H chain-associated idiotypes were unique among 14-15 different RF strongly suggests that the autoantibodies employ a number of different $V_H$ genes.

In conclusion, the synthetic CDR peptide-induced antiidiotypes reveal that 70% of monoclonal human IgM-RF share two light chain-associated CRI. In contradistinction, all five H chain CDR-associated idiotypes were private, among 14-17 monoclonal IgM-RF analyzed. Together, these results establish that the majority of IgM-RF share homologous $V_L$, but have heterogeneous $V_H$. It seems likely that one (or very few) $V_L$ genes, but a larger number of $V_H$ genes, are used to encode IgM-RF autoantibodies.

**Summary**

Recently, we have used synthetic peptides corresponding to the complementarity-determining regions (CDR) of Ig molecules to induce antiidiotypic antisera. Peptide PSH3, representing the third CDR of the IgM rheumatoid factor (RF) Sie heavy (H) chain, induced a private antiidiotype that reacted with only one out of five IgM-RF. Peptide PSL2, corresponding to the second CDR of Sie light (L) chain, induced an antibody against a crossreactive idiotype (CRI), expressed by 10 out of 12 human IgM-RF analyzed. Herein, we report that five additional antiidiotypic antibodies were generated by immunization with synthetic peptides identical to the third L chain CDR of IgM-RF Sie (PSL3), the second and third H chain CDR of IgM-RF Wol, and the second and third CDR of IgM-RF Pom. As analyzed by immunoblot assay, both anti-PSL3 and anti-PSL2 reacted with the majority of 16 IgM-RF. In contrast, all five antiidiotypes induced by the H chain peptides reacted only with the parent proteins, except anti-PSH3, which reacted weakly with one additional RF. These results suggest that one (or very few) $V_L$ gene(s), but a larger number of $V_H$ genes, are used to encode IgM-RF autoantibodies.
We thank Drs. J. D. Capra, G. Abraham, J. Johnson, H. Metzger, and E. Osserman for proteins, and Dr. A. Solomon for subtyping. We acknowledge the excellent assistance of the Basic and Clinical Research Department word processing center in the preparation of this manuscript.

Received for publication 8 April 1985.

References
1. Kunkel, H. G., H. M. Muller-Eberhard, H. H. Fudenberg, and T. B. Tomasi. 1961. Gamma globulin complexes in rheumatoid arthritis and certain other conditions. J. Clin. Invest. 40:117.
2. Winchester, R. J., V. Agnello, and H. G. Kunkel. 1970. Gamma globulin complexes in synovial fluids of patients with rheumatoid arthritis: Partial characterization and relationship to lowered complement levels. Clin. Exp. Immunol. 6:689.
3. Carson, D. A., J.-L. Pasquale, C. D. Tsoukas, S. Fong, S. F. Slovin, S. K. Lawrance, L. Slaughter, and J. H. Vaughan. 1981. Physiology and pathology of rheumatoid factors. Springer Semin. Immunopathol. 4:161.
4. Slaughter, L., D. A. Carson, F. C. Jensen, T. L. Holbrook, and J. H. Vaughan. 1978. In vitro effects of Epstein-Barr virus on peripheral blood mononuclear cells from patients with rheumatoid arthritis and normal subjects. J. Exp. Med. 148:1429.
5. Welch, M. J., S. Fong, J. H. Vaughan, and D. A. Carson. 1983. Increased frequency of rheumatoid factor precursor B lymphocytes after immunization of normal adults with tetanus toxoid. Clin. Exp. Immunol. 51:299.
6. Manheimer, A. J., C. Victor-Kobrin, K. E. Stein, and C. A. Bona. 1984. Anti-immunoglobulin antibodies. V. Age-dependent variation of clones stimulated by polysaccharide TI-2 antigens in 129 and MRL mice spontaneously producing anti-gamma-globulin antibodies. J. Immunol. 133:562.
7. Nemazee, D. A., and V. L. Sato. 1982. Enhancing antibody: A novel component of the immune response. Proc. Natl. Acad. Sci. USA. 79:3828.
8. Nemazee, D. A., and V. L. Sato. 1983. Induction of rheumatoid antibodies in the mouse: regulated production of autoantibody in the secondary humoral response. J. Exp. Med. 158:529.
9. Coulie, P., and J. Van Snick. 1983. Rheumatoid factors and secondary immune responses in the mouse. II. Incidence, kinetics and induction mechanisms. Eur. J. Immunol. 13:895.
10. Clarkson, A. B., and G. M. Mellow. 1981. Rheumatoid factor–like immunoglobulin M protects previously uninfected rat pups and dams from Trypanosoma lewisi. Science (Wash. DC). 214:186.
11. Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Cooper. 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti-gamma-globulin activity. J. Exp. Med. 137:331.
12. Andrews, D. W., and J. D. Capra. 1981. Complete amino acid sequence of variable domains from two monoclonal human anti-gamma globulins of the Wa cross-idiotypic group: suggestion that the J segments are involved in the structural correlate of the idiotype. Proc. Natl. Acad. Sci. USA. 78:3799.
13. Capra, J. D., and J. M. Keohoe. 1974. Structure of antibodies with shared idiotype: the complete sequence of the heavy chain variable regions of two immunoglobulin M anti–gamma globulins. Proc. Natl. Acad. Sci. USA. 71:4032.
14. Chen, P. P., R. A. Houghten, S. Fong, G. H. Rhodes, T. A. Gilbertson, J. H. Vaughan, R. A. Lerner, and D. A. Carson. 1984. Anti-hypervariable region antibody induced by a defined peptide. A new approach for studying the structural correlates of idiotypes. Proc. Natl. Acad. Sci. USA. 81:1784.
15. Chen, P. P., S. Fong, D. Normansell, R. A. Houghten, J. G. Karras, J. H. Vaughan, and D. A. Carson. 1984. Delineation of a cross-reactive idiotype on human autoantibodies with antibody against a synthetic peptide. *J. Exp. Med.* 159:1502.

16. Chen, P. P., F. Goni, S. Fong, F. Jirik, J. H. Vaughan, B. Frangione, and D. A. Carson. 1985. The majority of human monoclonal IgM rheumatoid factors express a primary structure–dependent cross-reactive idiotype. *J. Immunol.* 134:3281.

17. Kabat, E. A., T. T Wu, H. Bilofsky, M. Reid-Miller, and H. Perry. 1983. Sequences of Proteins of Immunologic Interest. U.S. Department of Health and Human Services, Washington, D.C.

18. Andrews, D. W., and J. D. Capra. 1981. Amino acid sequence of the variable regions of light chains from two idiotypically cross-reactive human IgM anti–gamma-globulins of the Wa group. *Biochemistry.* 20:5816.

19. Andrews, D. W., and J. D. Capra. 1981. Amino acid sequence of the variable regions of heavy chains from two idiotypically cross-reactive human IgM anti–gamma-globulins of the Wa group. *Biochemistry.* 20:5822.

20. Merrifield, R. B. 1963. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* 85:2149.

21. Houghten, R. A., W. C. Chang, and C. H. Li. 1980. Human beta-endorphin: synthesis and characterization of analogs iodinated and tritiated at tyrosine residues 1 and 27. *Int. J. Pept. Protein Res.* 16:311.

22. Liu, F.-T., M. Zinnecker, T. Hamaoka, and D. H. Katz. 1979. New procedures for preparation and isolation of conjugates of proteins and a synthetic copolymer of d-amino acids and immunochemical characterization of such conjugates. *Biochemistry.* 18:690.

23. Ledford, D. K., F. Goni, M. Pizzolato, E. C. Franklin, A. Solomon, and B. Frangione. 1983. Preferential association of kappa-IIIb light chains with monoclonal human IgM-kappa autoantibodies. *J. Immunol.* 131:1322.

24. Solomon, A., and C. L. McLaughlin. 1969. Bence-Jones proteins and light chains of immunoglobulins. II. Immunochemical differentiation and classification of kappa-chains. *J. Exp. Med.* 130:1295.

25. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350.

26. Chen, P. P., S. Fong, R. A. Houghten, and D. A. Carson. 1985. Characterization of an epibody: An antiidiotype which reacts with both the idiotype of rheumatoid factors (RF) and the antigen recognized by RF. *J. Exp. Med.* 161:323.

27. Laemelli, U. K. 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature (Lond.)*. 227:680.

28. Kunkel, H., D. Posnett, and B. Pernis. 1983. Anti-immunoglobulins and their idiotypes: Are they part of the immune network. *Ann. N. Y. Acad. Sci.* 418:324.

29. Gearhart, P. J., N. D. Johnson, R. Douglas, and L. Hood. 1981. IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. *Nature (Lond.)*. 291:29.

30. Bothwell, A. L. M., M. Paskind, M. Reth, T. Imanishi-Kari, K. Rajewsky, and D. Baltimore. 1981. Heavy chain variable region contribution to the NP(b) family of antibodies: Somatic mutation evident in a gamma-2a variable region. *Cell.* 24:625.

31. Crews, S., J. Griffin, H. Huang, K. Calame, and L. Hood. 1981. A single Vh gene segment encodes the immune response to phosphorylcholine: Somatic mutation is correlated with the class of the antibody. *Cell.* 25:59.

32. Kocher, H. P., C. Berek, and J. Jaton. 1981. The immune response of Balb/c mice.
to phosphorylcholine is restricted to a limited number of V(H)- and V(L)-isotypes. 
*Mol. Immunol.* 18:1027.

33. Rudikoff, S., M. Pawlita, J. Pumphrey, and M. Heller. 1984. Somatic diversification 
of immunoglobulins. *Proc. Natl. Acad. Sci. USA.* 81:2162.

34. Hartman, A. B., and S. Rudikoff. 1984. V(H) genes encoding the immune response 
to beta-(1,6)-galactan: Somatic mutation in IgM molecules. *EMBO (Eur. Mol. Biol. 
Organ.) J.* 3:5023.

35. Pons-Estel, B., F. Gőni, A. Solomon, and B. Frangione. 1984. Sequence similarities 
among kIIIb chains of monoclonal human IgMk autoantibodies. *J. Exp. Med.* 160:893.

36. Rajewsky, K., and T. Takemori. 1983. Genetics, expression, and function of idiotypes. 
*Ann. Rev. Immunol.* 1:569.

37. Capra, J. D., and M. Fougereau. 1983. Structural correlates of idiotypes. One from 
column A, one from column B. *Immunol. Today.* 4:177.

38. Sakano, H., K. Huppi, G. Heinrich, and S. Tonegawa. 1979. Sequences at the somatic 
recombination sites of immunoglobulin light-chain genes. *Nature (Lond.)*. 280:288.

39. Max, E. E., J. G. Seidman, and P. Leder. 1979. Sequences of five potential recombina-
nation sites encoded close to an immunoglobulin k constant region gene. *Proc. Natl. 
Acad. Sci. USA.* 76:3450.

40. Early, P., H. Huang, M. Davis, K. Calame, and L. Hood. 1980. An immunoglobulin 
heavy chain variable region gene is generated from three segments of DNA: V(H), 
D, and J(H). *Cell.* 19:981.

41. Sakano, H., R. Maki, Y. Kurosawa, W. Roeder, and S. Tonegawa. 1980. Two types 
of somatic recombination are necessary for the generation of complete immunoglob-
lulin heavy-chain genes. *Nature (Lond.)*. 286:676.

42. Sakano, H., Y. Kurosawa, M. Weigert, and S. Tonegawa. 1981. Identification and 
nucleotide sequence of a diversity DNA segment (D) of immunoglobulin heavy-chain 
genes. *Nature (Lond.)*. 290:562.

43. Siebenlist, U., J. V. Ravetch, S. Korsmeyer, T. Waldmann, and P. Leder. 1981. 
Human immunoglobulin D segments encoded in tandem multigenic families. *Nature 
(Lond.)*. 294:631.

44. Kurosawa, Y., and S. Tonegawa. 1982. Organization, structure, and assembly of 
immunoglobulin heavy chain diversity DNA segments. *J. Exp. Med.* 155:201.

45. Rudikoff, S., M. Pawlita, J. Pumphrey, E. Mushinski, and M. Potter. 1983. Galactan-
binding antibodies. Diversity and structure of idiotypes. *J. Exp. Med.* 158:1385.

46. Pawlita, M., M. Potter, and S. Rudikoff. 1982. K-chain restriction in anti-galactan 
antibodies. *J. Immunol.* 129:615.