SYNTHETIC PEPTIDES CORRESPONDING TO THIRD HYPERVARIABLE REGION OF HUMAN MONOCLONAL IgM RHEUMATOID FACTOR HEAVY CHAINS DEFINE AN IMMUNODOMINANT IDIOTYPE

By ROBERT D. GOLDFIEN, POJEN P. CHEN, SHERMAN FONG, AND DENNIS A. CARSON

From the Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California 92037

The structural basis of idiotypic antigens has been studied intensively in murine model systems (1), but less thoroughly in humans. Analyses of idiotype-antiidiotype reactions may be important in understanding the pathogenesis of human immunologic diseases, and in developing new approaches to treatment. Kunkel and coworkers (2), and subsequently Powell and Agnello (3), showed that IgM anti-IgG autoantibodies (rheumatoid factors [RF]) from unrelated individuals may display crossreactive idiotypes. The heavy and light chain variable region sequences of several IgM-RF have been reported (4–6). However, genetic studies and hybridoma techniques are difficult to apply in an outbred human population. Therefore, we have taken a different approach toward defining relationships between idiotype and structure on these human Ig. Rabbit antisera were generated against synthetic peptides with sequences corresponding to the hypervariable regions (complementarity determining regions [CDR]), of human IgM-RF. The antipeptide antibodies enabled us to clarify the structural basis for a private (7) and a crossreactive (8) idiotype on the anti-IgG autoantibodies.

Recent results in mice (9, 10), and our own preliminary data in humans (11), have focused attention on the third CDR of the heavy chain as a site of dominant idiotype formation. We now report that antisera raised against synthetic peptides corresponding to the third heavy chain CDR consistently recognize idiotypes expressed by intact IgM-RF autoantibodies. In contrast, high-titer antibodies against synthetic peptides representing the first and second heavy chain CDR infrequently bind to the IgM-RF molecule. These results suggest a distinctive role of the D (diversity-generating) region in the generation of idiotypic determinants, and have implications concerning the manipulation of idiotype-antiidiotype reactions in patients.

Materials and Methods

Preparation of Synthetic Peptides. The methods for peptide synthesis, coupling to keyhole limpet hemocyanin (KLH), and immunization of rabbits with the peptide-protein

TABLE I

Amino Acid Sequences of Peptides

Name*	Residues spanned[‡]	Amino acid sequence
1. PPH1	22–35	AASGFTFSSSAMSC[§]
2. PPH2	49–65	AWKYENGNDKHYADSVNG(GGC)[§]
3. PPH3	95–102	DAGPYVSPTFFAH(GGC)[§]
4. PSH1	22–35	KTSGGTFSGYTISC[‖]
5. PSH2	49–65	GSPAKWTDPFQGVYIKWE(GGC)[‖]
6. PSH3	95–102	EWKGQVNVNPFDY(GGC)[‖]
7. PWH2	49–65	GQIPLRFNGEVKNPGSVV(GGC)[‖]
8. PWH3	95–102	EYGFDTSDYYYYY(GGC)[‖]

* The abbreviation, PXHN, describes the peptides, P; X, the cognate protein (P, Pom; S, Sie; W, Wol); H, the heavy chain; and N denotes the first, second, or third CDR.

‡ Numbering is after Kabat et al. (16).

§ Sequences reported by Capra and Kehoe (4).

‖ Sequences reported by Andrews and Capra (5).

conjugates have been previously described in detail (7, 8). Briefly, the peptides were synthesized by Merrifield's solid-phase method (12), slightly modified (8). The derivation and sequences of the peptides are given in Table I. Rabbits were injected twice with the peptide-conjugates emulsified in complete Freund's adjuvant, and were boosted twice with glutaraldehyde-crosslinked peptides in incomplete Freund's adjuvant, as reported earlier (7). In some cases, rabbits were injected once with conjugates in complete Freund's adjuvant, and boosted with conjugates in incomplete Freund's adjuvant. Sera were obtained at biweekly intervals after immunization, and were stored in 0.02% sodium azide at −20°C.

Purification of Proteins.   Plasma or purified proteins were generously provided by Drs. J. D. Capra (Univ. Texas Health Sciences Center, Dallas, TX), G. Abraham (Univ. Rochester School of Medicine, Rochester, NY), J. Johnson (Nashville, TN), H. Metzger (National Institutes of Health, Bethesda, MD), and V. Agnello (Lahey Clinic, Burlington, MA). IgM cryoglobulins were prepared as described (11).

Enzyme-linked Immunosorbant Assay (ELISA).   The ELISA method for detecting antipeptide and anti-RF antibodies has been reported previously (8).

Immunoblotting.   Protein samples (20 μg each) were loaded onto 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate, and were electrophoresed at 30 mA for 3 h. The proteins were then transferred to nitrocellulose paper electrophoretically (70 V for 1.5–2 h). Protein binding sites on the papers were blocked with 5% powdered milk in borate-buffered saline (BBS). Rabbit antiserum, diluted in 2% powdered milk-BBS, was added, and incubated for 1 h. Finally, after extensive washing with BBS, the papers were incubated with ^{125}I-protein A (1 mCi/mg, 2 × 10⁵ cpm/ml), for 1 h, washed, and exposed overnight to Kodak XAR-5 film.

Results

Production of Antipeptide Antibodies.   The sequences of the synthetic peptides used are listed in Table I. Antibodies against each peptide-KLH conjugate were prepared in at least two rabbits, which were bled biweekly. All serum specimens were tested by ELISA for peptide-binding activity. Fig. 1 shows that, in each case, high titers of antipeptide antibodies were elicited. None of the antibodies bound significantly to an irrelevant peptide (PWL3). Pooled serum from nonimmune rabbits did not react substantially with the peptide-coated plates (data not shown). Thus, all the peptides studied were immunogenic. There was no apparent correlation between the sequence of the CDR peptide and the degree of immunogenicity.
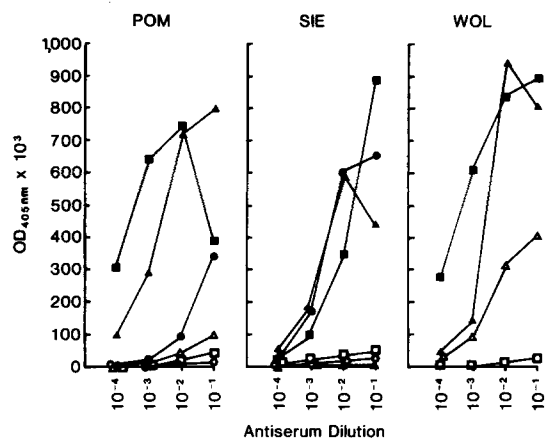
FIGURE 1. Reactivity of antipeptide antibodies with the immunizing peptides in ELISA. Plates were precoated with 10 μg/ml of peptides, and reacted with increasing dilutions of corresponding antisera, followed by alkaline phosphatase–labeled goat anti–rabbit antibody. The closed symbols represent binding of antisera to immunizing peptide: (●), CDR1; (■), CDR2; (▲), CDR3. The open symbols represent binding to the irrelevant peptide PWL3 (○), CDR1; (□), CDR2; (△), CDR3. Binding is expressed as optical density (OD) × 10^3 at 405 nm, at 30 min after addition of substrate. Points represent the mean of duplicate determinants, with <5% variability between replicates.

TABLE II

Reactivity of Antipeptide Antibodies with a Panel of Human IgM-RF by Immunoblotting

Antipeptide antiserum	IgM-RF antigen*																
	Pom	Sie	Wol	Les	Pay	Got	Glo	Neu	Gar	Cha	Sou	Mcd	Ark	Tal	Bel	Dri	Blo
PPH1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PPH2	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PPH3	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PSH1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PSH2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PSH3	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PWH2	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PWH3	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–

A (+) denotes reactivity of the antipeptide antibody with the RF, as detected by immunoblotting. A (–) indicates lack of reactivity.
* The IgM-RF Pom, Sie, Wol, Pay, Got, Glo, Neu, and Gar have been described (6). Cha, Sou, Mcd, Ark, Tal, Bel, Dri, and Blo were the generous gift of Dr. V. Agnello (3).

Reactivity of Antipeptide Antisera by Immunoblotting. The reactivity of the antipeptide antisera with isolated heavy chains of IgM-RF was analyzed by immunoblotting. The results are summarized in Table II. There are two points of note: (a) no antiserum reacted with more than a single RF, and (b) not all antisera reacted even with the isolated heavy chain of the RF containing the immunizing sequence. Specifically, the anti-PSH1 and anti-PSH2 antisera failed to bind to isolated Sie heavy chain. This has been a consistent result, despite repeated immunizations in several rabbits.

In every case, antisera directed against the third CDR sequence reacted more strongly with the isolated heavy chain than did antisera directed against the first or second CDR. This result is illustrated in Fig. 2, which compares the reactivity of each antipeptide antiserum with its respective RF heavy chain. Although anti-
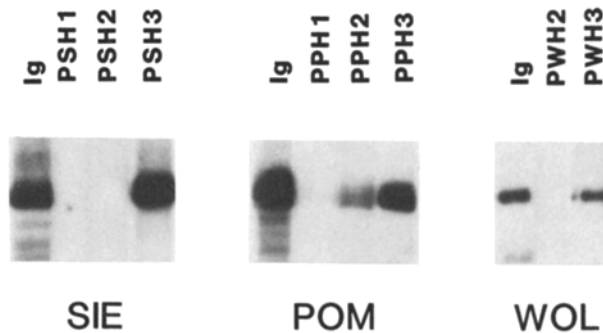
FIGURE 2.   Reactivity of antipeptide antibodies with isolated RF heavy chains by immuno-blotting. All antipeptide antisera were diluted 1:20. Binding was assessed by binding of ^{125}I-protein A binding, followed by autoradiography.
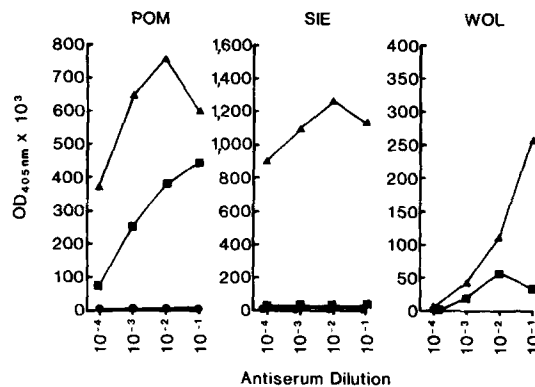

FIGURE 3.   Reactivity of antipeptide antibodies with intact RF by ELISA. Plates were coated with 2 μg/ml (Pom, Sie) or 10 μg/ml (Wol) and reacted with serial dilutions of antisera, followed by alkaline phosphatase–labeled goat anti–rabbit antibody (●), CDR1; (■), CDR2; (▲), CDR3. The values represent net OD × 10^3 at 450 nm between test sera and pooled normal rabbit serum at equivalent dilution, measured at 60 min after addition of substrate.

PPH1 and anti-PWH2 appear to be unreactive in this figure, they are in fact reactive with longer exposure (data not shown).

Binding of Antipeptide Antisera To Intact RF by ELISA.   The binding of the eight different antipeptide antibodies to intact IgM-RF was tested by ELISA, using microtiter plates coated with the purified RF proteins, and increasing concentrations of antipeptide antisera (Fig. 3). All three anti-CDR 3 antisera bound to the intact IgM-RF molecule. In contrast, of the five antibodies against CDR 1 and CDR 2 peptides, only anti-PPH2 reacted well with the corresponding IgM-RF protein. As noted earlier (Fig. 1), all antisera recognized specifically, and in high titer, the immunizing CDR peptide.

Discussion

We have assessed the ability of synthetic peptides, corresponding to heavy chain CDR sequences on human IgM-RF paraproteins, to elicit specific antiidi-otypic antibodies. At least two different rabbit antisera were generated against eight different synthetic peptide–KLH conjugates, representing all three heavy

chain CDR of two monoclonal IgM-RF (Sie, Pom), and the second and third CDR of an additional IgM-RF (Wol). In all cases, potent and specific antipeptide antibodies were produced. However, only the antisera against the third heavy chain CDR reacted consistently with the corresponding heavy chain polypeptides, and with the intact IgM-RF proteins. These results with synthetic peptides strongly suggest that the third heavy chain CDR sequence is associated with an immunodominant idiotypic determinant.

In the well-defined murine antidextran (13), antiphosphocholine (10), antigalactan (14), and antiarsonate (9) antibody systems, the heavy chain D region segment has been shown to play an important role in the generation of immunodominant idiotypes. The D region is responsible for the majority of the sequence variability in the third CDR of mouse heavy chains, but often contributes little to antigen binding specificity (13, 14). In this regard, it is interesting to note that the third heavy chain CDR may present an exposed, accessible surface on the Ig molecule, as predicted by computer modeling (14). In human heavy chains, the D region boundaries are less well defined (15, 16). However, it seems likely that the human D segment does constitute part of the third heavy chain CDR, and contributes to its variability. Conceivably, a major function of antibody D region gene segments may be to generate idiotypic diversity, independently of effects on antigen binding.

The ability of synthetic peptides to delineate the role of the heavy chain D segment, and the third CDR, in the formation of immunodominant idiotypes has clinical implications. Peptide-induced antiidiotypes provide a means for comparing the primary structures of Ig of unknown sequence. However, to be useful for the specific modulation of abnormal Ig production, the peptide-induced antibodies probably must recognize the intact cognate protein in its native form. Our results suggest that synthetic peptides corresponding to the third CDR of human heavy chains can reproducibly elicit antiidiotypic antibodies against intact human IgM-RF paraproteins.

Summary

Synthetic peptides corresponding to eight individual heavy chain complementarity-determining regions (CDR) of three human monoclonal IgM anti-IgG (rheumatoid factor [RF]) paraproteins elicited rabbit antibodies with markedly different properties. All antisera recognized the immunizing peptide, and several reacted with the isolated IgM heavy chain on immunoblots. However, only the antisera against peptides representing the third CDR bound consistently and specifically to the intact IgM-RF molecule. These data indicate that the third CDR of human μ chains comprises an immunodominant idiotype, and suggest that the D gene segment may be especially important in creating idiotypic diversity. Synthetic peptides corresponding to the third heavy chain CDR of human paraproteins may be clinically useful for the specific induction of antiidiotypic antibodies.

Karras for excellent technical assistance, and the BCR Word Processing Center for typing the manuscript.

References

1. Rudikoff, S. 1983. Immunoglobulin structure-function correlates: antigen binding and idiotypes. *Contemp. Top. Mol. Immunol.* 9:169.
2. Kunkel, H. G., V. Agnello, F. G. Joslin, and J. D. Capra. 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti–gamma-globulin activities. *J. Exp. Med.* 139:128.
3. Powell, R., and V. Agnello. 1983. Characterization of complement-fixing activity and cross-idiotypes of rheumatoid factors in idiopathic mixed cryoglobulinemia. *Clin. Immunol. Immunopathol.* 29:146.
4. Capra, J. D., and J. M. Kehoe. 1974. Structure of antibodies with shared idiotype: The complete sequence of the heavy chain variable regions of two immunoglobulin M anti-gammaglobulins. *Proc. Natl. Acad. Sci. USA.* 71:4032.
5. 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.
6. Ledford, D. K., F. Goni, M. Pizzolato, E. C. Franklin, A. Solomon, and B. Frangione. 1983. Preferential association of kappa-III-b light chains with monoclonal human IgM-kappa autoantibodies. *J. Immunol.* 131:1322.
7. Chen, P. P., R. A. Houghten, S. Fong, G. H. Rhodes, T. A. Gilberston, J. H. Vaughan, R. A. Lerner, and D. A. Carson. 1984. Anti-hypervariable region antibody induced by a defined peptide: an approach for studying the structural correlates of idiotypes. *Proc. Natl. Acad. Sci. USA.* 81:1784.
8. Chen, 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 antibodies with antibody against a defined peptide. *J. Exp. Med.* 159:1502.
9. Gridley, T., M. N. Margolies, and M. L. Gefter. 1985. The association of various D elements with a single immunoglobulin Vh gene segment: influence on the expression of a major cross-reactive idiotype. *J. Immunol.* 134:2, 1236.
10. Berek, C. 1984. The D segment defines the T15 idiotype. The immunoresponse of A/J mice to pneumococcus pneumoniae. *Eur. J. Immunol.* 14:1043.
11. Chen, P. P., F. Goni, R. A. Houghten, S. Fong, R. Goldfien, J. H. Vaughan, B. Frangione, and D. A. Carson. Characterization of human rheumatoid factors by seven anti-idiotypes induced by synthetic hypervariable-region peptides. *J. Exp. Med.* 162:487.
12. Merrifield, R. B. 1963. Solid Phase Peptide, I. The Synthesis of a tetrapeptide. *J. Am. Chem. Soc.* 85:2149.
13. Clevinger, B., J. Shilling, L. Hood, and J. M. Davie. 1980. Structural correlates of cross-reactive and individual idiotypic determinants on murine antibodies to alpha-(1 → 3) dextran. *J. Exp. Med.* 151:1059.
14. 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.
15. Siebenlist, U., J. V. Favetch, S. Korsmeyer, T. Waldman, and P. Leder. 1981. Human immunoglobulin D segments encoded in tandem multigenic families. *Nature (Lond.).* 294:631.
16. Kabat, E. A., T. T. Wu, H. Bilofsky, M. Reid-Miller, and H. Perry. 1983. Sequences of proteins of immunological interest. U. S. Department of Health and Human Services, Bethesda, MD.