INVERTEBRATE RECOGNITION PROTEIN CROSS-REACTS WITH AN IMMUNOGLOBULIN IDIOTYPE

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To determine the minimal structural requirements for shared idiotypy, we tested the lectin of the horseshoe crab, Limulus polyphemus, for its capacity to react with monoclonal antibodies directed against idiotypic determinants on the phosphorylcholine-binding murine myeloma protein TEPC-15. This model was chosen because the Limulus lectin binds phosphorylcholine as well as sialic acid (1) and shows a number of similarities to the acute-phase protein C-reactive protein (CRP)[1] that binds phosphorylcholine (2) and expresses determinants cross-reactive with the idiotypes of phosphorylcholine-binding myeloma proteins (3). The structure of horseshoe crab lectin is considerably different from that of vertebrate immunoglobulins (4–6). Moreover, Limulus, an ancient arachnoid, is a "living fossil" that has had an evolution completely distinct from that of the vertebrates (7). We assessed the capacity of monoclonal antibodies specific for idiotypic determinants of the TEPC-15 molecule (3, 8, 9) to bind to horseshoe crab lectin in the presence or absence of phosphorylcholine derivatives or sialic acid. We report that the lectin cross-reacts with TEPC-15 and CRP using defined monoclonal antibodies and we identify short stretches of amino acid sequence implicated in the cross-reaction and in the binding of hapten.

Materials and Methods

Invertebrate Lectins and CRP. Limulin (lectin from Limulus polyphemus) was either obtained from EY Labs, San Mateo, CA or prepared (10) from Limulus serum kindly supplied by Dr. J. Granberry, Limulus Laboratories, FL. Didemnum candidum lectin I (DC-I) was purified as reported earlier (11). Bovine serum albumin (BSA), bovine submaxillary mucin (BSM), N-acetylneuraminic acid (NANA) type VI, phosphorylcholine chloride (Ca^{++} salt) (PC), p-nitrophenylphosphorylcholine (pNPPC), and human α_1-acid glycopro-

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; BSM, bovine submaxillary mucin; CRP, C-reactive protein; DC-I, Didemnum candidum lectin I; ELISA, enzyme-linked immunoadsorbent assay; Id, idiotype; MAb, monoclonal antibody; NANA, N-acetylneuraminic acid; PC, phosphorylcholine; pNPPC, p-nitrophenylphosphorylcholine; Rbα-CRP, rabbit anti-CRP antiserum; VH, variable region heavy chain.

tein were purchased from Sigma Chemical Co., St. Louis, MO at the highest purity available. The myeloma protein TEPC-15 was purchased from Litton Bionetics, Inc., Kensington, MD. Human CRP was prepared as reported elsewhere (12). We are grateful to Dr. J. E. Volanakis, University of Alabama, Birmingham, AL for the generous gifts of human CRP and rabbit anti-CRP antisera (Rb α-CRP).

Monoclonal Antibodies (MAb). Anti-TEPC-15 idiotype (Id) MAb 4C11 and F6 were produced as described earlier (9) and purified on TEPC-15-Sepharose as reported elsewhere (13). The production and characterization of all other anti-TEPC-15 Id MAb tested in the present report has been described previously (8). MAb specific for anti-α_{1-3} dextran idiotypic determinants were obtained as previously reported (14). MAb DB1-1, GB4-10, B2-44, and AB1-2 to TEPC-15 and all antidextran Id were the generous gift of Dr. J. F. Kearney, University of Alabama, Birmingham.

Enzyme-linked Immunoadsorbent Assay (ELISA). The reactivity of mAB for myeloma and acute-phase proteins and lectins was assessed by an ELISA carried out in accord with our previously published procedures (15). When capabilities of certain molecules (NANA, PC, pNPPC, BSM) to inhibit the binding of MAb to test proteins was examined, 50 µl of the inhibitor solutions in Tris-buffered saline (100 mM Tris HCl, 50 mM NaCl, 10 mM CaCl$_2$, pH 7.6) were incubated on the coated plates for 45 min at room temperature. 50 µl of the MAb (5 µg/ml) serial dilutions were added to the wells and incubated for another 45 min. Plates were washed and the procedure was followed as described (15).

Results

MAb specific for CRP, TEPC-15 idiotype, and antidextran idiotypes showed diverse cross-reactivities (Table I) when tested in ELISA with the acute-phase protein CRP (which binds PC), the myeloma protein TEPC-15 (which binds PC), and the serum lectin (limulin) from the American horseshoe crab *Limulus polyphemus*, specific for sialoconjugates and also reported to bind PC (1). MAb of anti-CRP specificity cross-reacted with TEPC-15 but not with limulin, whereas antidextran Id-specific MAb were negative for all proteins tested, as was the MAb specific for insulin that was tested as negative control. All anti-TEPC-15 Id-specific MAb showed strong reactions with TEPC-15 and many of them cross-reacted intensely with limulin and CRP. The antiserum Rb α-CRP also cross-reacted with limulin. Other proteins that were tested, the acute phase α_1-acid glycoprotein and the lectin DC-I, from the tunicate *Didemnum candidum*, specific

TABLE I

Reactivity of Lectins, Acute-Phase Proteins and Myeloma Proteins with MAb and Rabbit Anti-CRP Antibodies

Antibody code	Specificity	Reactivity (ELISA)*		
		CRP	TEPC-15	Limulin
AB1-2	Anti-T15 Id (needs light + heavy	+	+	−
GB4-10	chain)	±	+	±
B24-44	Anti-T15 Id⁺	+	+	+
DB1-1	Anti-T15 Id⁺ specific (made against isolated T15 heavy chain)	+	+	+
F6	Anti-T15 Id	+	+	+
4C11	Anti-T15 Id	+	+	+
Rb α-CRP	Anti-CRP	+	+	+

* α_1-Acid glycoprotein (human), DC-I, and BSA were negative for all MAb and Rb α-CRP. All antidextran Id MAb tested were negative with CRP, TEPC-15, limulin, α_1-acid glycoprotein, DC-I, and BSA.

for galactosyl residues, did not show any cross-reactivity with the MAb and neither did BSA, included as a negative control.

The strongest cross-reactivities with limulin were observed with the MAb DB1-1 (anti-TEPC-15 variable region heavy chain (V_H) specific, made against the isolated TEPC-15 heavy chain), F6 (anti-TEPC-15 Id), and 4C11 (anti-T15 Id). F6 and 4C11 titrated almost identically with CRP and limulin, as shown in Fig. 1, where titration curves for TEPC-15, DC-I, and BSA are also shown.

We confirmed the specificity of the anti-TEPC-15 Id MAb and the nature of the binding site recognized on CRP and limulin by testing the binding of F6 and 4C11 MAb in the presence of PC, pNPPC, NANA, and BSM alternatively. Titration curves (Fig. 2) of MAb F6 and 4C11 tested with limulin and CRP were not modified with respect to the buffer controls by the presence of NANA (100 mM) or BSM (100 μg/ml). However, the intensity of the reaction was strongly decreased when the titration was performed in the presence of pNPPC (100 mM). The sodium salt of PC (100 mM) was a less powerful inhibitor than the p-nitrophenyl derivative.
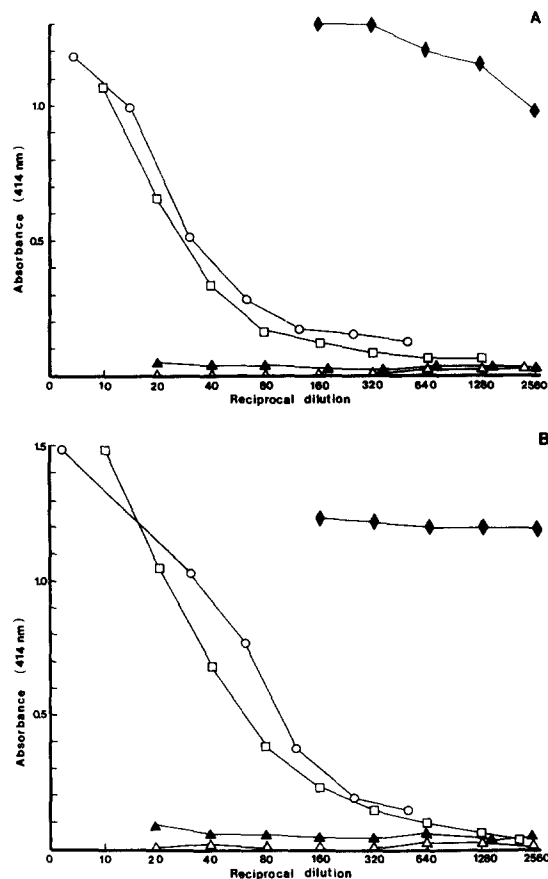

FIGURE 1. Binding by ELISA of MAb directed against idiotypic determinants of the TEPC-15 immunoglobulin to limulin and CRP. MAb tested: 4C11 (A) and F6 (B). Proteins: TEPC-15 (◆), CRP (□), limulin (○), D. candidum lectin (▲), and BSA (△).
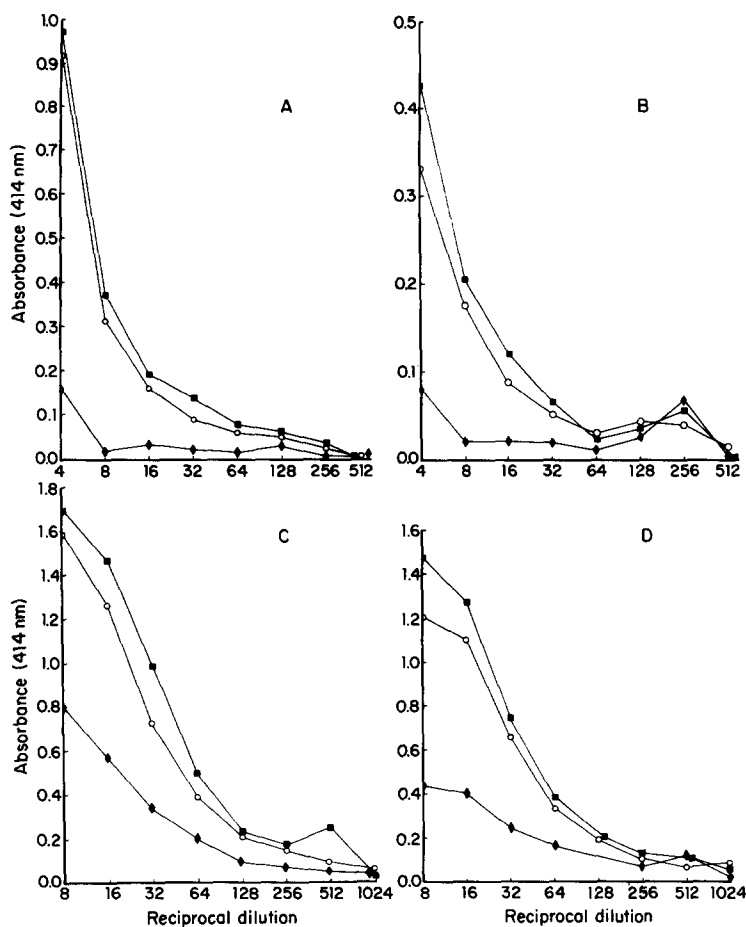
FIGURE 2.   Inhibition assay to determine the effects of NANA and pNPPC on the binding of MAb F6 and 4C11 directed against TEPC-15 idiotypic determinants to limulin and CRP. MAb F6 is used in A and C, 4C11 in B and D. Limulin is tested in A and B, CRP in C and D. No inhibitor (PBS control) (■); NANA (O); pNPPC (◆).

Discussion

Our results show that the lectin of an ancient arachnoid bears a serological determinant detectable using MAb directed against an antigen-specific Id of a murine myeloma protein. Using rabbit antisera directed against human CRP, we showed that the horseshoe crab lectin is cross-reactive with CRP. Using MAb specific for the TEPC-15 Id, we found that some of these (DB1-1, F-6, and 4C11) showed clear reactivity with both horseshoe crab lectin and CRP. The horseshoe crab lectin shares "idiotopes" with TEPC-15 but it is clearly not equivalent to TEPC-15 Id because all of the MAb we tested bound strongly to the immunoglobulin, whereas many MAb to TEPC-15 or to related V_H structures did not react with the limulin molecule. The capacity of the MAb to react with horseshoe crab lectin was diminished by using pNPPC, indicating that the anti-Id and the hapten were competing for the combining site. Free sialic acid or sialoproteins, by contrast, did not block the binding of MAb to limulin. There-

fore, the binding sites for sialic acid and PC are distinct on the lectin molecule and are probably spacially distant, accounting for the lack of cross-inhibition.

Neither CRP (16) nor limulin (6) show significant overall sequence homology to vertebrate immunoglobulins. We used the computer program RELATE (17) to consider sequence stretches of 6 and 10 amino acids to identify short regions of significant sequence identity that might account for the observed serological cross-reaction between CRP and limulin and the binding of MAb to the TEPC-15 Id of both molecules. The only stretch of significant identity between the sequenced portion of limulin and CRP corresponded to 59–71 in limulin and 52–64 in CRP. 9 of 13 positions were identical within this stretch, as follows:

Limulin	F S Y A T A K K D N E L L
CRP	F S Y A T K R Q D N E I L

The application of the same analysis to a comparison of TEPC-15 and CRP identified a stretch of CRP sequence corresponding to residues 83–92 of CRP and residues 60–69 of TEPC-15. This short stretch showed 50% identity of residues. A comparison between TEPC-15 and limulin identified a stretch of sequence that overlapped the one identified in the TEPC-15/CRP sequence. The stretch corresponded to residues 64–71 of TEPC-15 and 47–54 of limulin. This limited stretch of sequence likewise showed 50% identity. These sequences are illustrated as follows:

TEPC-15	S A S VK G R F I V S R
Limulin	K G T L G H S R
CRP	S A S GI V E F W V

Although the shared residues are not identical in the comparisons of limulin and CRP with TEPC-15, these stretches overlap and the binding of MAb anti-Id can be blocked by hapten. The region of TEPC-15 V_H identified corresponds to the juncture of the second complementarity-determining region (CDR2) and the third framework (FR3) segments. Additional evidence that this CDR2/FR3 sequence stretch is critical is the fact that monoclonal anti-TEPC is GB4-10 which reacts with D region sequence (18) and binds only marginally to CRP and limulin and that the proposed region identified here is potentially an exposed antigen site, on the basis of computer analysis of hydrophilicity (19). The lengths of shared sequence, correlated with shared antigenicity and ligand binding, correspond in size (6–15 residues) to that stated as optimal for the production of antipeptide antibodies that react with the intact protein (20).

Although it might be concluded that the sharing of relatively short stretches of sequence between classic immunoglobulins and nonimmunoglobulin molecules most probably illustrates a convergent evolution in which different molecules reactive with a common ligand are forced to use similar residues to form the combining site, another interpretation that might account for the sharing of idiotype (21) as well as the definition of V_H allotype (22) is the insertion of short DNA segments or minigenes (23) into different framework sequences. Our results are clearly pertinent to the use of antibodies directed against idiotypic immunoglobulin Id determinants as probes for receptors on lymphocytes or on antigen-specific factors, because they indicate that only a very short stretch of

sequence is required to allow sharing of combining site-related idiotopes among totally unrelated molecules.

Summary

To estimate the minimal structural requirements for cross-reaction of idiotypic determinants, we determined the capacity of monoclonal antibodies specific for the idiotype of the phosphorylcholine (PC)-binding myeloma protein TEPC-15 for cross-reactivities with the PC-binding, acute-phase protein C-reactive protein (CRP) and the hemagglutinin from the horseshoe crab *Limulus polyphemus* (limulin), which binds sialic acid and PC. Certain monoclonal antibodies (MAb) to the TEPC-15 idiotype showed strong cross-reactions with CRP and limulin when tested by enzyme-linked immunoadsorbent assays. The specificity of the cross-reactivities was confirmed by testing the binding of the reactive anti-TEPC-15 MAb to both CRP and limulin in the presence of *p*-nitrophenylphosphorylcholine (*p*NPPC), *N*-acetylneuraminic acid, and bovine submaxillary mucin. The binding of the MAb to both CRP and limulin was strongly decreased by *p*NPPC, partially decreased by free PC, and not affected by *N*-acetylneuraminic acid or bovine submaxillary mucin. Neither CRP nor limulin showed significant overall sequence homology to vertebrate immunoglobulins. However, CRP, limulin, and TEPC-15 variable region heavy chain (V_H) shared short stretches of homology (8–10 amino acids) that mapped to a stretch comprised of the second complementarity determining region and third framework region of the TEPC-15 V_H. These results might reflect either evolutionary convergence forced upon molecules of diverse evolutionary histories because of steric requirements of binding the same ligand, or a conservation of primitive combining site gene segments in evolution.

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