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THE ROLE OF A NOVEL VH SEQUENCE (V11) IN THE FORMATION OF ANTI-PHOSPHOCHOLINE ANTIBODIES*

JACQUELINE W. PIERCE, SUSAN HUDAK and J. LATHAM CLAFLIN
Program in Cellular and Molecular Biology and Department of Microbiology and Immunology, The University of Michigan Medical School, Ann Arbor, MI 48109, U.S.A.

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Abstract—The immune response to phosphocholine (PC) in mice is highly restricted. Most anti-PC antibodies use heavy-chain variable-region (VH) sequences derived from single VH gene segment, *V1*. In order to investigate whether a highly homologous VH gene segment, *V11*, could contribute to the formation of PC-binding antibodies, we carried out chain recombination experiments with M47A, a non-PC binding myeloma protein whose H-chain is encoded by the *V11* gene segment, and two PC-binding antibodies, HP101.6G6 (HP6G6) and M511. The H-chains from the non-PC-binding myeloma protein, M47A, formed a functional PC-binding site when paired with L-chains from both PC-binding antibodies. These results suggest that a second VH gene segment, *V11*, could theoretically be used to form PC-binding antibodies. In addition, these results provide direct evidence that a single H-chain can be used in combinatorial association with different L-chains to form antibodies of differing specificities.

INTRODUCTION

The role of germline, somatic and combinatorial mechanisms is generating immunoglobulin diversity has been studied extensively in the antibody response to PC† (Crews *et al.*, 1981., Gearhart *et al.*, 1981; Selsing and Storb, 1981). The response to this small hapten is highly restricted (Claflin and Rudikoff, 1976) and sequence information on murine myeloma and hybridoma proteins has shown that most anti-PC antibodies use a single type of H-chain sequence (VH4) which is paired with one of three different types of L-chain sequences (VK8, VK22 or VK24) (Barstad *et al.*, 1978). Crews *et al.* (1981) identified a family of four highly homologous VH segments in BALB/c mice (designated *V1*, *V11*, *V13* and *V3*), and they have clearly shown that most anti-PC antibodies use VH sequences that are somatically derived from a single member of this VH gene family, *V1*. Recently, however, several observations have suggested that other members of this VH family (*V11* or *V13*) might be used to generate H-chains of PC-binding antibodies. Clarke *et al.* (1982) reported the sequence of a monoclonal anti-PC antibody of CBA/J origin, HP101.6G6 (HP6G6), which appears to be derived from the CBA/J homologue of *V11*. A second example of an anti-PC antibody derived from a gene other than *V1* is the BALB/c hybridoma protein HPCG15, which has an *N*-terminal sequence that is more closely related to V11 or V13 than it is to V1 (Gearhart *et al.*, 1981). There is no known example of an anti-PC antibody that uses the precise V11 or V13 germline sequence. The question we wished to address was whether the germline V11 VH sequence could contribute to the formation of anti-PC antibodies. Specifically, could an H-chain having the germline V11 sequence pair with an appropriate L-chain to form a functional PC-binding site? An H-chain which has the V11 germline sequence has been observed in the non-PC-binding myeloma protein M47A (Robinson and Appella, 1979). In order to examine the possible role of V11 sequences in the formation of a PC-binding site, we carried out chain recombination experiments between the H-chain of M47A and L-chains from two PC-binding antibodies.

The prediction that the M47A H-chain might be able to participate in formation of a PC-binding site was based on several observations. While the M47A VH sequence contains substitutions which are located at or near the so-called "contact residues" (Padlan *et al.*, 1976), many of these substitutions (positions 30, 32, 35 and 51) have been observed in the PC-binding protein HP6G6 and thus are not incompatible with PC-binding (see Fig. 1). The entire H-chain variable region of M47A is notably similar to the VH region of HP6G6. The M47A sequence differs from HP6G6 at seven positions in the VH segment. The M47A DH segment is the same length as that of HP6G6 and the

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†Abbreviations: BSA, bovine serum albumin; H, heavy; L, light; PC, phosphocholine; PC–BGG, PC–bovine gamma globulin; *V1* and V1, gene and gene product of a VH segment.

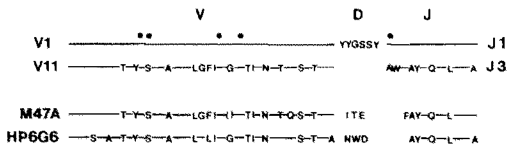

Fig. 1. Comparison of complete VH region sequences of M47A and HP6G6 with BALB/c germline encoded sequences. The translated sequences of BALB/c gene segments *V1* and *V11* (top two lines) given in single-letter amino acid code were determined by Crews *et al.* (1981). The translated DH sequence (Kurosawa and Tonegawa, 1982), J1 sequence (Early *et al.*, 1980) and J3 sequence (Gough and Bernard, 1981) are also shown. Solid line indicates identity with the translated V1 or J1 sequence. Amino acid sequence of M47A VH region (Robinson and Appella, 1979) and HP6G6 VH regions (Clarke *et al.*, 1982) (bottom two lines) are compared with the BALB/c germline encoded sequences. Substitutions from V1 and J1 are noted. Contact residues according to Padlan *et al.* (1976) are indicated with a solid circle.

M47A JH sequence is encoded by the same JH gene as the HP6G6 JH sequence (JH3). In these experiments, the M47A H-chain was paired with either HP6G6 L-chain or M511 L-chain. Since the M47A VH sequence is most closely related to the HP6G6 VH sequence, we predicted that the HP6G6 L-chain would be the most likely L-chain to pair with M47A H-chain. The M511 L-chain was selected because it has an *N*-terminal sequence identical to HP6G6 L-chain (Clarke S., personal communication). Our goal was to determine whether M47A H-chains could form a functional PC-binding site with either of these L-chains. We measured PC-binding activity in H- and L-chain mixtures and not the precise extent of H–L reassociation because the requirements for reconstitution of binding activity are more rigorous than the requirements for formation of a stable recombinant molecule (Barstad *et al.*, 1978; Bridges and Little, 1971; Kranz and Voss, 1981; Manjula *et al.*, 1976). We show that the V11-derived M47A H-chain formed a functional PC-binding recombinant when paired with L-chains from both HP6G6 and M511.

MATERIALS AND METHODS

Myeloma and hybridoma proteins

The myeloma cell line M47A was obtained from Litton Bionetics Inc. (Kensington, MD). M47A urinary protein was purified from the urine of BALB/c mice (Jackson Laboratory, Bar Harbor, ME) bearing subcutaneous M47A tumors as described (Bevan, 1971). Urine was clarified by centrifugation (300 *g*), concentrated with sucrose and dialyzed extensively against distilled water and then against 0.04 *M* potassium phosphate buffer (pH 8). The M47A protein was then purified by elution from a DEAE-Sephadex column (1.5 × 55 cm) in 0.04 *M* potassium phosphate buffer (pH 8) as described by Askonas and Williamson (1968). The PC-binding myeloma protein, M511, and hybridoma protein HP6G6 were purified from tumor ascites by affinity chromatography on PC-Sepharose as described (Claflin and Cubberly, 1980).

Anti-idiotypic antiserum: rabbit anti-M47A

A heterologous anti-idiotypic antiserum was made against the purified myeloma protein M47A. A rabbit was immunized intradermally (i.d.) with 1 mg M47A in CFA. Then, 2 weeks later, it received a secondary immunization of 1 mg M47A in saline (i.d.). Serum was obtained 8 days after secondary immunization. The gamma globulin fraction was precipitated with 40% ammonium sulfate and then dialyzed against PBS. The antiserum was then absorbed by passage over a Sepharose immunoadsorbent column containing BALB/c normal mouse serum (NMS). The BALB/c NMS had been previously absorbed on PC-Sepharose to remove naturally occurring anti-PC antibodies. The anti-M47A was also absorbed on a Sepharose column to which M511, M167 and M603 myeloma proteins had been coupled. Absorption of antiserum on these columns was designed to remove activity directed against NMS, the α constant region, the kappa constant region and any variable-region determinants on M511, M167 or M603. The specificity of this antiserum was tested by direct binding of radiolabelled myeloma proteins and by inhibition assay with radiolabelled M47A.

H- and L-chain separation

H- and L-chains were prepared from purified myeloma or hybridoma proteins as described by Bridges and Little (1971). A 1-ml protein solution containing 50 mg was dialyzed against 0.2 *M* Tris–Cl (pH 8.2), 0.15 *M* NaCl and 0.002 *M* Na_2EDTA. Each protein was reduced with 0.01 *M* dithiothreitol for 2 hr and alkylated with 0.022 *M* iodoacetamide for 15 min. This reduction and alkylation procedure was performed twice. Samples were then dialyzed against 6 *M* urea and 1 *N* acetic acid solvent before separation of H- and L-chains by reverse flow chromatography on a G100 Sephadex column (2.5 × 100 cm) in the urea–acetic acid solvent. The M47A H-chain (mol. wt 40,000) and L-chain (mol. wt 23,000) were separated on a larger G100 Sephadex column (2.5 × 170 cm). Separated M47A H-, M47A L-, M511 H- and M511 L-chains were stored at 4°C in 6 *M* urea and 1 *N* acetic acid until used in chain recombination experiments. The HP6G6 L-chain was dialyzed against 0.01 *M* ammonium bicarbonate buffer and stored as lyophilized protein. Purified H- and L-chains were tested for purity by SDS-polyacrylamide gel electrophoresis (SDS–PAGE) as described by Laemmli (1970). The M47A H-, M511 H- and M511 L-chain samples were visualized by silver staining as described by Wray *et al.* (1981). M47A L- and HP6G6 L-chains were visualized on a separate gel by staining with 0.01% Coomassie blue.

Chain reassociation

Chain reassociation was performed as described by Bridges and Little (1971). H- and L-chains were mixed in 6 *M* urea and 1 *N* acetic acid at an H:L absorbancy ratio (280 nm) of approximately 1:1 as indicated. Final protein concn was 50–200 μg/ml. Reassociation was achieved by gradual stepwise dialysis of mixed chains against distilled water then PBS at 4°C. Control preparations of H-chain alone or L-chain alone were treated similarly.

Solid-phase radioimmunoassay (SRIA)

Direct binding assay. Chain recombination mixtures were tested for their ability to bind ^{125}I-labelled PC–BGG in an SRIA. PC–BGG was prepared as described (Claflin and Davie, 1975) and iodinated by the procedure of Greenwood *et al.* (1963). H-chains, L-chains or H + L chain mixtures were used to coat polyvinyl chloride microtiter wells (Dynatech Laboratories, Alexandria, VA) (0.2 ml/well) for 6 hr at 25°C at concns indicated. Protein was aspirated and wells were washed 3 times with PBS. Wells were then saturated with 1% BSA in PBS for 1 hr. A 0.2 ml solution of ^{125}I-labelled PC-BGG (50,000 cpm) in 1% BSA, PBS was added and wells were incubated for 12–14 hr at 25°C. Unbound PC–BGG was removed by aspiration and wells were washed 3 times with PBS. The binding of ^{125}I-PC–BGG to protein-coated wells was assayed by counting individual wells in a Packard Auto-Gamma Scintillation Spectrometer. Duplicates were run for each binding assay.

Inhibition assay for idiotype. Rabbit anti-M47A antiserum at a 1:1000 dilution was used in a solid-phase inhibition assay as described by Claflin and Davie (1975). Binding of ^{125}I-labelled M47A (50,000 cpm/ml) in the presence of unlabelled inhibitor protein was measured.

RESULTS

Purification of H- and L-chains

The G100-Sephadex separation resulted in quantitative yields of H- and L-chains from each parent, including M47A, indicating that the unusual covalent bond between M47A H- and L-chains (Robinson and Appella, 1979) was efficiently reduced under these conditions. Results of an SDS–polyacrylamide gel of M47A H, M511 H and M511 L are shown in Fig. 2. Titration of each sample at several concns showed

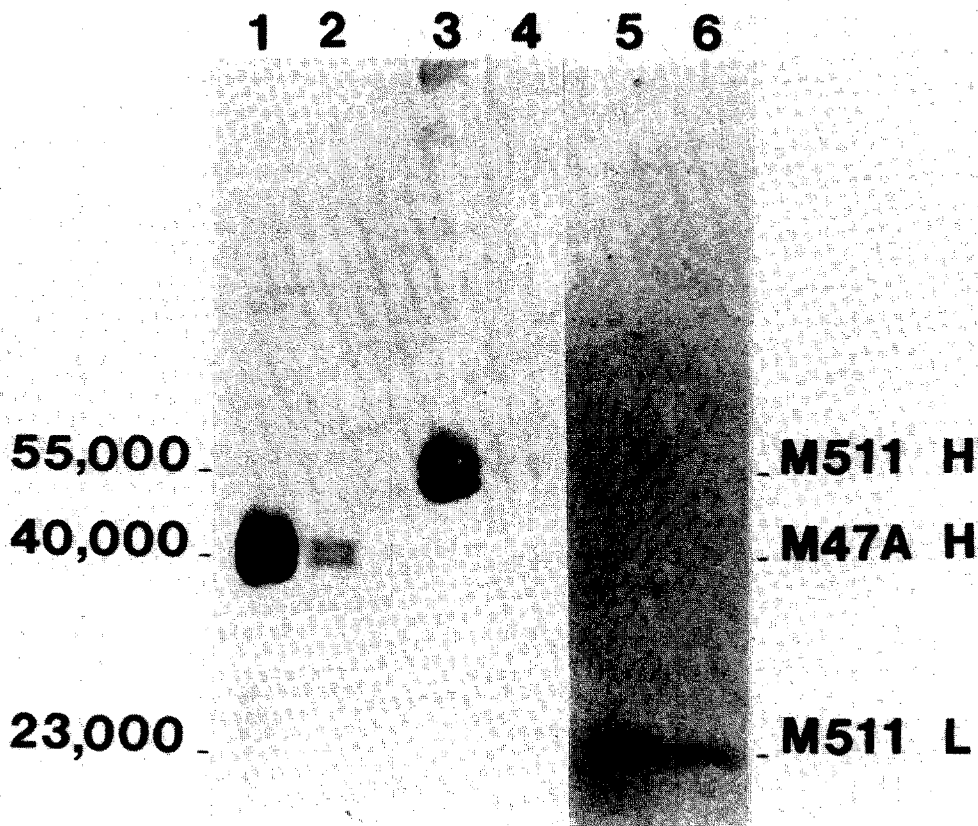

Fig. 2. SDS–PAGE analysis of separated H- and L-chains. Separated H- and L-chains were analyzed in a 10% polyacrylamide gel containing 0.1% SDS and 8 *M* urea. Gel was run for 5 hr at 20 mA. Polypeptides were visualized using silver-staining procedure as described by Wray *et al.* (1981). Lane 1, M47A H: 5 μg; lane 2, M47A H: 1 μg; lane 3, M511 H: 8 μg; lane 4, M511 H: 1.5 μg; lane 5, M511 L: 1 μg; and lane 6, M511 L: 0.2 μg.

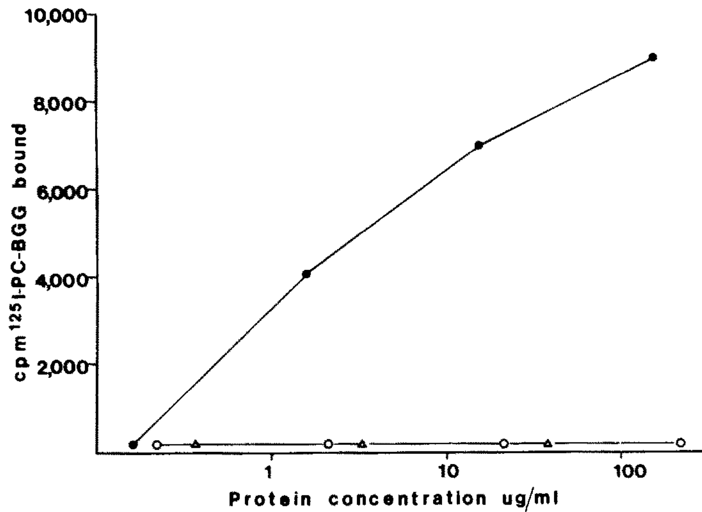

Fig. 3. Homologous recombination of M511 H and M511 L: direct binding of ^{125}I-PC–BGG. M511 H-chains and M511 L-chains were mixed while in urea–acetic acid solvent at an H:L absorbance ratio at 280 nm of 1:1. Final protein concn after dialysis of H + L mixture was 163 μg/ml. Homologous mixture (H + L), M511 H or M511 L at concns indicated were tested for the ability to bind ^{125}I-PC–BGG in a direct SRIA as described in Materials and Methods. Each point is the average of duplicate determinations. M511 H + M511 L (●——●), M511 H (○——○), M511 L (△——△).

that individual chains contained less than 1% contamination. The M47A H-chain had an apparent mol. wt of approximately 40,000 which is consistent with previous observations of Robinson and Appella (1979) who found that the M47A H-chain lacked the CH3 domain. Preparations of M47A L, HP6G6 H and HP6G6 L had less than 5% contamination as estimated from Coomassie blue stained SDS–PAGE (data not shown).

Homologous chain mixtures

The ability of separated chains to recombine and form functional molecules was first tested by mixing homologous chains, i.e. H- and L-chains derived from the same parental molecule. The M511 H-chain was mixed with M511 L-chain while in urea–acetic acid solvent, dialyzed and then tested for the ability to bind ^{125}I-PC–BGG as described in Materials and Methods. In these experiments, we were principally concerned with the reconstitution of functional binding sites and not the extent of H–L association. Results of homologous recombination are shown in Fig. 3. The separated chains did not demonstrate any detectable binding activity. Thus, the purified chains did not contain any intact molecules or contaminating chains that could yield a detectable recombinant upon dialysis. The level of binding of the mixture of M511 H + M511 L was approximately 80% of the binding activity observed for the reduced and alkylated parent molecule. Thus, separated M511 H- and L-chains were capable of efficient recombination to form a functional PC-binding site.

Separated H- and L-chains from the myeloma protein M47A were then tested for their ability to form a homologous recombinant. Since the M47A protein has no known binding activity, the ability to recombine was tested by the reconstitution of M47A idiotypic determinants. The anti-M47A idiotypic antiserum used to monitor recombination reacted exclusively with M47A and showed no activity toward M511 (IgA, κ) or HP6G6 (IgM, κ) which possess CH, CK or VH regions similar to M47A (see Fig. 4). The homologous recombination mixture of M47A H + M47A L inhibited binding of ^{125}I-M47A (native) to this anti-idiotypic antiserum, and the level of inhibition was the same as that observed with a reduced and alkylated preparation of the parent M47A. Individual M47A H-chains or M47A L-chains showed some ability to inhibit; however, they had 4- to 18-fold lower activity, respectively, in this assay. These results demonstrate that the mixing of M47A H- and L-chains is accompanied by a reconstitution of idiotypic determinants indicating that M47A H and M47A L are able to recombine to form a molecule which is idiotypically similar to intact M47A.

Heterologous chain mixtures

To determine whether M47A H-chain, containing V11-derived sequences, could form a functional recombinant with the L-chain from a PC-binding antibody, the M47A H-chain was paired with either the HP6G6 L-chain or the M511 L-chain. The first step in the heterologous recombination experiments was to demonstrate that the HP6G6 L-chain was functional. Homologous recombination between HP6G6 H and HP6G6 L did not result in formation of a functional molecule due to the insolubility of the HP6G6 H-chain. As an alternative approach, a preliminary experiment was performed which combined

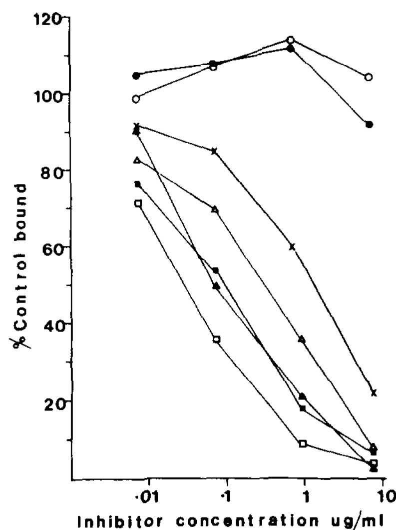

Fig. 4. Homologous recombination of M47A H and M47A L: expression of M47A idiotypic determinants. Inhibition SRIA as described in Materials and Methods was used to examine the expression of M47A idiotypic determinants on individual M47A H-chains, M47A L-chains or M47A H + L mixture. M47A H-chains were mixed with M47A L-chains in urea–acetic acid solvent at an H:L absorbance ratio at 280 nm of 1:2. Final protein concn in H + L mixture after dialysis was 65 μg/ml. The binding of ^{125}I-M47A (50,000 cpm/ml) to anti-M47A at 1:1000 was inhibited by unlabelled proteins as follows; HP6G6 (○——○), M511 (●——●), M47A H-chain (×——×), M47A L-chain (△——△), M47A H + M47A L (■——■), reduced + alkylated M47A (▲——▲), intact M47A (□——□).

HP6G6 L-chain with M511 H-chain. This H-chain, unlike HP6G6 H-chain, retains its solubility when separated from the parent molecule. The result of heterologous recombination of M511 H and HP6G6 L is shown in Fig. 5A. The H + L mixture showed significant PC-binding activity while individual H- or L-chains did not show any detectable binding. The activity observed with this heterologous recombination was similar to that seen in the homologous recombination of M511 H with M511 L (compare Figs 5A and 3).

When M47A H was paired with HP6G6 L-chain under identical experimental conditions a slightly different result was obtained (Fig. 5B). Individual H- or L-chains alone, treated the same as H + L mixtures, showed no detectable activity. When M47A H-chains were mixed with HP6G6 L-chains, PC-binding activity was reconstituted. The control experiment of M47A H + M47A L did not show any PC-binding activity, indicating that the HP6G6 L-chain was necessary for the reconstitution of a functional binding site. It is important to note, however, that the level of PC-binding which was reconstituted in the heterologous mixture of M47A H + HP6G6 L is less than 10% of the activity observed when M511 H-chain was used under identical conditions (compare Fig. 5A and B).

Further experiments pairing M47A H-chain with M511 L-chain gave results similar to those obtained with M47A H + HP6G6 L (see Fig. 5C). The PC-binding activity for the M47A H + M511 L mixtures was comparable to that of M47A H + HP6G6 L. The ratio of H:L (1:1 or 2:1) had no apparent effect on the reconstitution of binding activity.

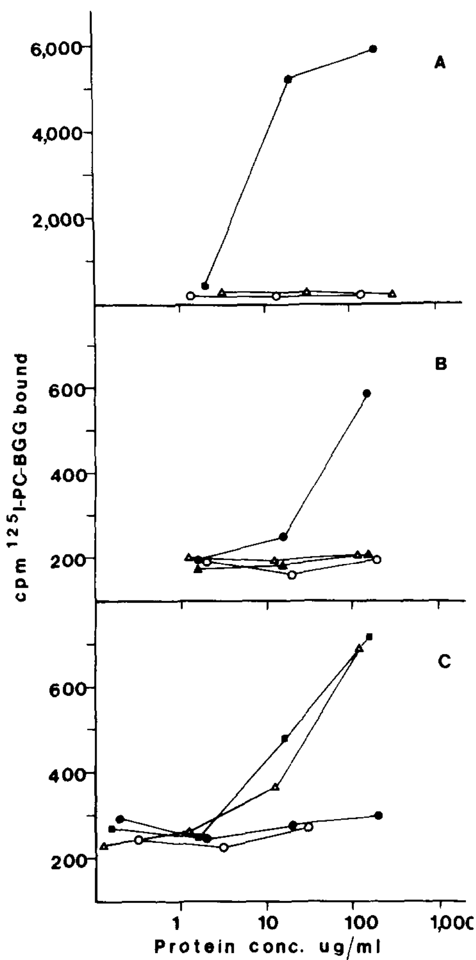

Fig. 5. Heterologous recombinations: direct binding of ^{125}I-PC–BGG. Direct SRIA binding assay was used to examine the binding of ^{125}I-PC–BGG (250,000 cpm/ml) to individual H-chains, L-chains or H + L mixtures. Background counts bound in the absence of protein sample were approximately 200 cpm. Total cpm bound are indicated. (A) M511 H + HP6G6 L: M511 H-chains were mixed with HP6G6 L-chains at an H:L absorbance ratio at 280 nm of 1:1. Final protein concn after dialysis was 200 μg/ml. M511 H + HP6G6 L (●——●), M511 H (△——△), HP6G6 L (○——○). (B) M47A H + HP6G6 L: M47A H-chains were mixed with HP6G6 L-chains at an H:L absorbance ratio of 1:1. Final protein concn after dialysis was 173 μg/ml. M47A H + HP6G6 L (●——●), HP6G6 L (△——△), M47A H (○——○), M47A H + M47A L (▲——▲). (C) M47A H + M511 L: M47A H-chains were mixed with M511 L-chains at an H:L absorbance ratio of either 1:1 or 2:1. Final protein concn was 125–180 μg/ml. M47A H + M511 L, 1:1 (△——△); M47A H + M511 L, 2:1 (■——■); M47A H (●——●), M511 L (○——○).

DISCUSSION

The goal of these experiments was to determine whether the germline *V11* sequence could participate in the formation of a PC-binding antibody. Our results show that the M47A H-chain, which contains the V11 germline sequence, is able to form a PC-binding site when paired with either HP6G6 L-chains or M511 L-chains. These data suggest that a second member of the PC VH gene family, *V11*, could theoretically be used to form PC-binding antibodies. Thus, the observation that most anti-PC antibodies use V1 or V1-derived sequences and not the V11 sequence cannot be attributed to the inherent inability of the V11 sequence to cooperate in formation of a PC-binding site. The question of why the *V11 germline* sequence is not used to encode anti-PC antibodies cannot be answered simply. One likely possibility is that antibodies using V11 sequences may have a much lower affinity for PC than those that use V1 sequences. This might be predicted from the observation that HPCG 15, which appears to use a non-V1 sequence, has an affinity for PC which is 10-fold lower than most anti-PC antibodies (Gearhart *et al.*, 1981). A second reason why V11 germline sequences are not found in anti-PC antibodies may be that the V11 sequence does not pair efficiently with the appropriate VK sequence. The precise sequence requirements for VH–VL pairing have not been established. In murine anti-PC antibodies, Rudikoff *et al.* (1982) suggest that a glutamic acid residue at position 35 of the H-chain may interact with tyrosine at position 94 of the L-chain. These investigators noted that the *V1* gene is the only member of this VH family encoding glutamic acid at position 35. The fact that the V11 and V13 VH sequences have a glutamic acid residue to serine substitution at this position could affect the ability of these VH sequences to pair efficiently with an appropriate L-chain; however, this serine substitution occurs in the proteins HP6G6 and HPCG15, indicating that this substitution does not preclude H–L interaction. Preferential use of the *V1* gene product to encode anti-PC antibodies may reflect the importance of residue 35 in the structure of the PC-binding site.

While our data show that the M47A chain is capable of forming a PC-binding site, they provide only limited information on the relative effectiveness of the V11 germline sequence compared to V1 in formation of PC-binding antibodies. We observed that heterologous mixtures of M47A H-chain (V11) with HP6G6 L-chain showed less than 10% of the PC-binding activity seen in a heterologous mixture of M511 H-chain (V1) with the same L-chain. This lower binding activity, however, may be due to several factors. Chain recombination experiments involving the M47A H-chain are complicated by the fact that the M47A myeloma protein is an IgA half-mer (one H-chain and one L-chain), it lacks the CH3 H-chain domain and it contains an unusual covalent bond between the H- and L-chain (Mushinski, 1971; Robinson and Appella, 1979). The lack of a CH3 domain should not affect the ability of M47A H to pair with the L-chain since only VH–VK and CH1–CK interactions are involved in H–L pairing (Alexandru *et al.*, 1980; Klein *et al.*, 1979). However, recombination involving the M47A H-chain would be expected to result in the formation of two-chain molecules rather than the usual four-chain molecules. In these experiments, we have not determined whether the lower binding activity observed with M47A H-chain mixtures reflects less efficient H–L reassociation or the formation of a heterologous recombinant with a lower affinity for PC. It is likely that both decreased H–L pairing and lower affinity would be observed. The per cent H–L reassociation observed might be influenced by the stability of two-chain molecules and by possible trace amounts of homologous M47A L-chain in the M47A H-chain sample that could compete with heterologous L-chain (Grey and Mannik, 1965). The fact that M47A H and HP6G6 L recombinants exist as two-chain molecules should not affect the affinity of the binding site (Zack *et al.*, 1981); however, monovalency may account for reduced binding observed in SRIA due to reduced avidity of these antibodies. Two-chain molecules cannot be affinity purified on PC–Sepharose (Zack *et al.*, 1981). Thus, we could not study the affinity of M47A H + HP6G6 L recombinant molecules or the idiotypic properties of this heterologous recombinant because we were unable to purify sufficient material for these experiments.

Our data provide new information on the role of germline, somatic and combinatorial mechanisms in generating antibody diversity. The observation that the V11 sequence could be used in anti-PC antibodies suggests that germline VH gene segments other than *V1* may play a role in the immune response to PC. Other investigators have reported that PC-binding antibodies which use non-V1 sequences (VH12) can be found in T15-suppressed mice (Kocher *et al.*, 1980) and that a subset of T15 idiotype-negative antibodies in the memory response to PC–KLH may use a VH sequence different from V1 (Chang *et al.*, 1982; Wicker *et al.*, 1982). It would be interesting to investigate whether suppression of antibodies which utilize V1-encoded sequences would result in the use of the less common anti-PC antibodies from the V11 gene or other VH genes.

It is also possible that the germline *V11* sequence is not observed in the anti-PC response because substitutions in the V11 sequence (similar to that seen in HPCG15 or HP6G6) may be required for the formation of a functional anti-PC antibody from this germline sequence. Somatic diversification has been suggested to account for increased affinity of IgG anti-PC antibodies (Rodwell *et al.*, 1983). A similar finding has recently been reported for anti-arsonate antibodies (Rothstein and Gefter, 1983). It is possible that complementary substitutions in both VH and VL may be required for efficient PC-binding.

The role of combinatorial association in generating antibody diversity has been studied for many years. The chain recombination experiments presented here provide direct evidence for the role of combinatorial association in generating antibodies of different specificities. A single H-chain, M47A H, can pair with either M47A L or HP6G6 L to form antibodies with different specificities. Previous investigators have shown that the formation of functional antibodies through reassociation of heterologous H- and L-chains is quite restricted (Bridges and Little, 1971; Grey and Mannik, 1965; Kranz and Voss, 1981; de Preval and Fougereau, 1976). Functional heterologous recombinants have been formed between H- and L-chains derived from antibodies having highly homologous amino acid sequences. For example, heterologous recombination among functionally-related antibodies such as anti-galactan (Manjula *et al.*, 1976), anti-inulin (Streefkerk *et al.*, 1978), anti-H-2 (Bluestone *et al.*, 1982) or anti-PC (Barstad *et al.*, 1978; Hudak and Claflin, manuscript in preparation; Scher *et al.*, 1971) antibodies can result in the formation of functional recombinant molecules. Recently, several observations have suggested that the same VH sequence may contribute to the formation of antibodies with different specificities. An example of overlapping VH repertoires may be seen in the anti-streptococcus group A carbohydrate antibody S8 which has an *N*-terminal VH sequence very similar to the V1-encoded sequence of PC-binding antibodies (Capra *et al.*, 1976; Crews *et al.*, 1981). In addition, Staudt and Gerhard (1983) have recently reported that antibodies which bind the hemagglutinin (HA) protein of influenza may use sequences derived from the *V11* VH gene, indicating that similar VH regions may be used by both anti-HA and anti-PC antibodies. By contrast, the VK sequences of anti-HA antibodies are very different from the VK sequences of anti-PC antibodies indicating that the L-chain is critical in determining specificity of these antibodies. Previous studies (Leon and Young, 1971; Andres *et al.*, 1981; Hudak and Claflin, manuscript in preparation) have shown a correlation between L-chain type and fine specificity of PC-binding. The L-chain may contribute some contact residues or L-chain may influence conformation of the H-chain and thus dictate specificity indirectly (Kabat, 1981). Chain recombination experiments between anti-HA antibodies and anti-PC antibodies may prove useful in further delineating the role of combinatorial association in generating antibody diversity.

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