J.L. Claflin

Department of Microbiology, University of Michigan Medical School, Ann Arbor

Uniformity in the clonal repertoire for the immune response to phosphorylcholine in mice*

A comparison of the clonal nature of the immune response to phosphorylcholine (PC) was made in nine different inbred mouse strains. Quantitative idiotypic analysis showed that anti-PC antibodies from each strain were composed of antibodies bearing binding-site idiotypic determinants indistinguishable from two different BALB/c myeloma proteins, T15 and M511. Idiotypic determinants of two other PC-binding proteins, M167 and M603, were not detected. Isoelectric focusing of the light (L) chains verified the presence of antibodies similar to T15 and M511 in each strain and indicated the presence of two additional antibodies, one of which has an L chain which cofocuses with M603. Fractionation of anti-PC antibody with anti-idiotypic antibody showed that immunoglobulins bearing T15 and M511 idiotypic determinants are separate and contain L chains that are uniform and resemble those of T15 and M511, respectively.

Thus, these mice which differ genetically at multiple loci including the heavy chain allotype complex locus each possess, at least in part, an equivalent set of clonotypes specific for PC. This indicates that the genes encoding these antibodies must be contained in the germ line.

1. Introduction

Previous work from this laboratory has demonstrated that mouse strains, regardless of their genetic background, produce an antibody to phosphorylcholine (PC) which possesses a specificity [1] and binding-site idiotype (ID), $T15_s$ [2], indistinguishable from those of TEPC 15, a PC-binding myeloma protein originating in BALB/c. This suggests [1] a conservation of at least one clonotype throughout mouse strains even though genetic variants of this clonotype exist, such as the T15 ID [2, 3]. The finding of a PC-binding myeloma protein in CB 20 mice, BALB/c. B6 (IgG_H), supports this contention [4]. This myeloma protein carries the allotypic and T15 idiotypic determinant (IDD) like C57BL/6 antibody, but has a combining specificity, binding-region antigenic determinant, $T15_s$, and certain structural features which are remarkably similar to TEPC 15.

The present study confirms our earlier finding of the regular expression of $T15_s$ ID in different strains of mice. They extend those studies by showing that the $T15_s$ ID is present only on immunoglobulins which also resemble T15 in structure. In addition we will show that another anti-PC antibody, prototyped by MOPC 511, a different PC-binding myeloma protein, is also preserved throughout inbred mice.

[I 1461]

* This investigation was supported by the National Institutes of Health through grant AI-12533.

Correspondence: J. Latham Claflin, Department of Microbiology, The University of Michigan, 6643 Medical Science Building II, Ann Arbor, Michigan 48109, USA

Abbreviations: **BSA:** Bovine serum albumin **IEF:** Isoelectric focusing **PC:** Phosphorylcholine **TBA:** Tube binding assay **PBS:** Phosphate buffered saline **ID:** Idiotype(s), idiotypic **IDD:** Idiotypic determinants **DNP:** 2,4-Dinitrophenyl

2. Materials and methods

2.1. Purification of myeloma protein and anti-PC antibodies

The origin and maintenance of the PC-binding plasmacytomas TEPC 15, MOPC 167, HOPC 8, MOPC 511, McPC 603, and the 2,4-dinitrophenyl (DNP)-binding MOPC 460 have been described in detail elsewhere [4]. All mouse strains were obtained from Jackson Laboratories, Bar Harbor, Maine. Antisera were pooled from 10–20 mice given multiple intravenous (i.v.) injections of 10^8 heat-killed (56 °C, 30 min) *Streptococcus pneumoniae* strain R36A [5]. Antibodies and myeloma proteins with specificity for PC were isolated from a PC-Sepharose column by affinity chromatography [2]. The standard procedure [2] was modified in order to include a wash with 0.05 M N-carbobenzoxyglycine prior to elution of the specific protein with 10^{-2} M PC. This step, which removes a large quantity of nonspecifically adhering serum protein, pI 5.7 ± 0.5 [4], was found necessary whenever the serum concentration of specific antibody was less than 1–2 mg/ml. Isolated mouse anti-PC antibodies were shown to be of the IgM class by immunoelectrophoresis and by immunodiffusion with class-specific antisera. Antibodies in some instances were radiolabeled with ^{125}I [6].

2.2. Isoelectric focusing (IEF) of light chains

Purified myeloma proteins and anti-PC antibodies were first completely reduced with 0.2 M 2-mercaptoethanol in 7 M guanidine in Tris-HCl buffer, pH 8.2 (1 h, 23 °C) and alkylated with 0.4 M iodoacetamide (45 min, 4 °C) [7]. Heavy (H) and light (L) chains, separated on Sephadex G-100 in 1 M propionic acid–4.5 M urea, were analyzed by IEF in polyacrylamide gel slabs [4] or 0.3 x 12 cm tubes [8]. After focusing, slabs or tube gels were fixed and stained with Coomassie brilliant blue as described by Williamson [9].

2.3. Quantitation of IDD

Rabbit antibodies with specificity for the binding site of T15, anti-$T15_s$, have been described [10]. Guinea pig antibodies

having specificity for the binding site of M603, M511 and M167, (*i.e.* anti-M603$_S$, M511$_S$, M167$_S$) were prepared in a similar fashion [10]. Each ID antibody was completely specific for the respective myeloma protein; in both direct and indirect radioimmunoassays (see below) no cross-reactions were observed. Moreover, the reaction between each anti-ID antibody and its corresponding myeloma protein was greater than 90 %, inhibited by PC and was dependent on an H-L chain interaction [10].

Quantitation of IDD in anti-PC antibodies was examined by solid-phase radioimmunoassay (tube binding assay, TBA) as previously described [2]. The amount of any ID in an anti-PC antibody preparation was determined by comparing its inhibition to that of a standard titration of myeloma protein for each of the 4 ID anti-ID assay systems. The sensitivities of the assays ranged from 0.01 to 0.05 μg/ml.

2.4. Fractionation of individual ID (Scheme 1)

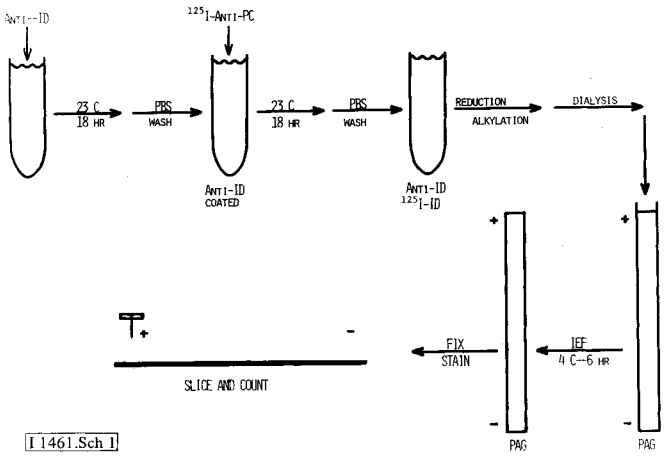
Purified ^{125}I-labeled antibody (2 x 10^5 – 2 x 10^6 cpm) in 2.5 ml of 0.15 M NaCl, 0.005 M phosphate buffered saline (PBS), pH 7.0, containing 1 % bovine serum albumin (BSA) was added to 10 x 75 mm glass tubes previously coated with anti-ID antibody (anti-ID), *e.g.* anti-T15$_S$. After 18 h incubation at 22 °C, the solution containing unreacted anti-PC antibodies was removed and the tube washed 3 times with PBS. The anti-ID-coated tube containing a bound antibody (ID) was completely reduced and alkylated as described above. A sample of ^{125}I-labeled anti-PC antibody taken prior to the adsorption experiments was completely reduced and alkylated and served as a reference. After focusing the L chains of each preparation (10 000 to 50 000 cpm) in tubes of polyacrylamide gel, each gel was sliced and each 2-mm piece was counted in a Packard gamma counter. Purified L chains of T15, M511 and M603 were added as markers, but their presence did not affect the results.

3. Results

3.1. IEF patterns of anti-PC L chains

Initial attempts to separate the IgM anti-PC antibodies by IEF in liquid columns [11] failed to resolve differences that might exist. Anti-PC antibodies from BALB/c, C57BL/6 and C58 each focused between pH 4.2 and 5.5 with a broad peak at pH 4.9 to 5.0. However, no fraction within this range was significantly enriched for a particular ID even though different ones were present (see below). Analysis of purified F(ab')$_2$ [12] and 7 S IgM$_S$ subunits [13] by disc gel electrophoresis and IEF revealed heterogeneity, but the majority of this could be accounted for by sialic acid residues, *i.e.* altered by treatment with neuraminidase. A first assessment of the degree of heterogeneity in anti-PC antibodies from different strains was therefore determined after IEF of isolated L chains, a procedure which we have shown readily reveals compositional differences as well as similarities in L chains of the PC-binding myeloma proteins [4]. Fig. 1 shows the stained L chain banding patterns of anti-PC antibodies from 8 representative strains and compares them to the L chains of three PC-binding and one DNP-binding myeloma proteins. The L chains of M167 and H8 are not shown since they focus in positions identical to those of M511 and T15, respectively [4]. The L chains of each of the myeloma proteins appear in

Scheme 1. Scheme for isolation of specific ID from a heterogeneous mixture of ID.



a set of closely grouped bands which is indicative of a homogeneous polypeptide [4, 14]. By contrast the L chain banding patterns of most of the mouse anti-PC antibodies were complex and ranged from pH 4.7 to 8.2. However, it is evident that bands corresponding in position to those of T15, M511 (M167), and M603 are present in all strains except BALB/c. In this antibody preparation, and in that taken at different times after immunization, BALB/c primarily expressed antibody with this T15 set of bands. C58 and CE L chains possess additional bands at pH 5.9, 6.4, 6.8 and 7.2, none of which cofocus with any bands from M511, M603, or T15.

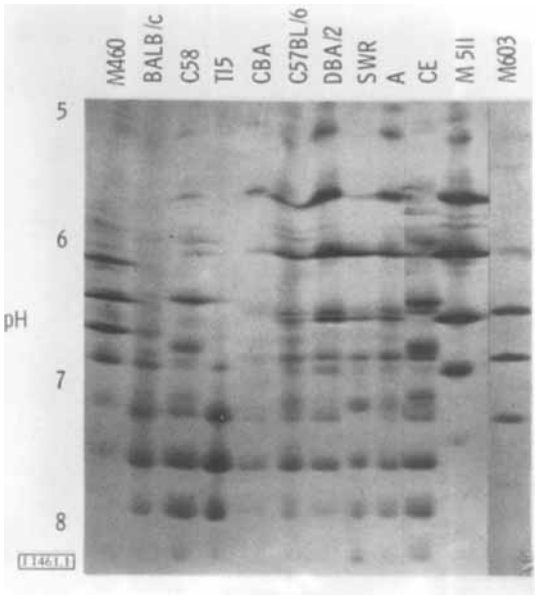


Figure 1. IEF patterns of L chains of anti-PC antibodies and of PC-binding myeloma proteins. IEF was carried out in a thin-layer polyacrylamide gel slab in pH 5 to 9.5 ampholytes. M603 was focused in a different portion of the gel, then placed adjacent to the other patterns with the proper alignment retained. Bands in the BALB/c pattern focusing in the range pH 6 to 6.8 were found in separate assays to result from contamination by M460.

In Fig. 2 the L chain patterns of C58 and DBA/2 depicted in Fig. 1 are analyzed. The L chains of these two mouse strains contain between them all the bands observed in any other strain of mice. In C58, for example, a mark for every band seen in the gel is placed adjacent to the slice. Subtrac-

tion of those representative of T15 (L_i) and M511 (L_{iv}) leaves a single set of closely spaced bands (L_{iii}). Similarly, after subtraction of T15- and M511-like bands from DBA/2, a single set of closely spaced bands is left (L_{ii}) which, in this case, cofocus with M603. Though we cannot be certain, the symmetrical displacement and identical disposition of L_{iii} in both C58 and CE suggest that it represents a single L chain type.

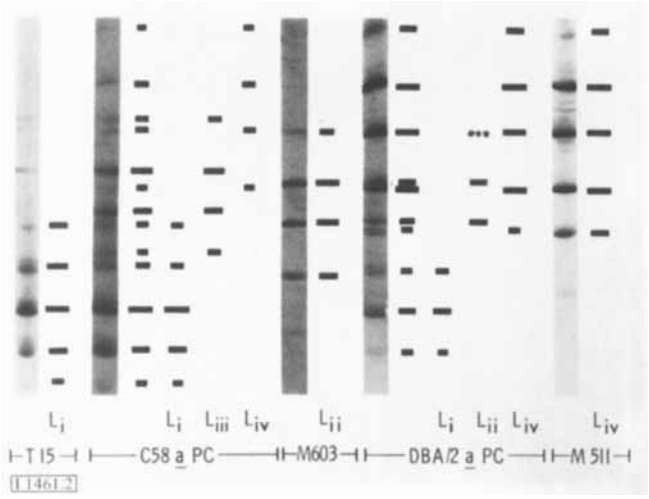


Figure 2. Analysis of L chain patterns in C58 and DBA/2 anti-PC antibodies shown in Fig. 1 by comparison with L chains of T15, M603 and M511.

3.2. Quantitation of ID

A portion of the pools of anti-PC antibodies from BALB/c, C58, C57BL/6, DBA/2, A and CE mice characterized in Fig. 1 were quantitated for IDD characteristic of the combining regions of the available PC-binding myeloma proteins. As shown in Table 1, each anti-ID antibody was specific for its corresponding myeloma protein and was capable of accurately recognizing that immunoglobulin in a pool of ID. Binding-site determinants of T15, $T15_s$ ID, were found in all strains tested but comprised as little as 12 % of total antibody in DBA/2 and as much as 100 % in BALB/c. $M511_s$ ID were readily detected in all strains with the surprising exception of BALB/c. Negligible amounts of ID with M603 and M167 characteristics were observed in every strain even though the assays were capable of measuring these ID at a concentration of 10–50 ng/ml. Separate assays performed with additional guinea pig and rabbit antibody preparations to binding-site as well as nonbinding-site regions [10] of M603 (3 antisera) and M167 (4 antisera) failed to reveal Ig bearing variable region similarities to M603 and M167. Furthermore, attempts to detect immunoglobulins which shared cross-reactive determinants [15, 16] with M167 or M603 (anti-M167 or anti-M603 vs. ^{125}I-labeled A/J anti-PC antibody) were unsuccessful. However, though the sum of $T15_s$- and 511_s-bearing immunoglobulins accounted for the majority of the anti-PC antibody present, in no instance except BALB/c did it account for all the ID in the sample. Thus, antibodies bearing binding-site structural features of two different BALB/c myeloma proteins are regularly expressed in inbred strains of mice and comprised the majority of the response to PC.

3.3. ID fractionation of anti-PC antibodies

To test the heterogeneity of antibodies having a particular ID and to determine the structural relation of this ID to the relevant myeloma protein, anti-PC ID were fractionated with anti-ID antibodies and examined for their L chain type as outlined in Scheme 1. The results obtained from fractionation of C57BL/6, AKR and CE anti-PC antibodies with anti-$M511_s$ and anti-$T15_s$ are shown as examples (Fig. 3). The positions in the gel occupied by M511 and T15 carrier L chains are depicted at the top of each panel. L chains of ^{125}I-labeled anti-PC antibodies, shown in the upper one-third of each panel, exhibit the multiple-banding pattern indicative of a heterogeneous population of L chains (Fig. 1). However, antibody specifically bound on anti-$M511_s$-coated tubes (middle portion of panel) contain L chains that migrate in a limited pH range, and the position of each peak of radioactivity corresponds precisely with those of the marker M511 L chains. Similarly, that anti-PC antibody reactive with anti-$T15_s$ contains four peaks of activity in its L chains which cofocus with those of the T15 L chains. Identical results, not shown

Table 1. Concentrations of immunoglobulins having different determinants in anti-PC antibodies

Strain	Ig[a] (μg/ml)	IDD[b] $T15_s$ (μg/ml)	$T15_s$ (%)	$M511_s$ (μg/ml)	$M511_s$ (%)	$M167_s$ (μg/ml)	$M167_s$ (%)	$M603_s$ (μg/ml)	$M603_s$ (%)
BALB/c	55	62[c]	112[c]	5	9	< 0.01	0	< 0.05	0
C58	600	218	35	162	27	< 0.01	0	< 0.05	0
C57BL/6	55	16	29	21	38	< 0.01	0	< 0.05	0
DBA/2	580	70	12	244	42	< 0.01	0	< 0.05	0
A	36	11	30	14	38	< 0.01	0	< 0.05	0
AKR	65	55	84	7	11	< 0.01	0	< 0.05	0
CE	250	69	28	75	30	< 0.01	0	< 0.05	0
Myeloma									
T15, M511, M603	33, each	34	34	29	29	< 0.01	0	30	30
T15, M167	50, each	44	44	< 0.01	0	48	48	< 0.05	0

a) Ig concentration of purified antibody.
b) Antibodies directed to binding site of H8 ($H8_s$), M511 ($M511_s$), M603 ($M603_s$), M167 ($M167_s$) were used.
c) Concentration of anti-PC antibody (% of Ig) giving 50 % inhibition of binding of ^{125}I-labeled myeloma protein to the corresponding anti-ID antibody was compared to a standard curve obtained with the appropriate myeloma protein. Error for each determination is ± 20 % of calculated concentration.

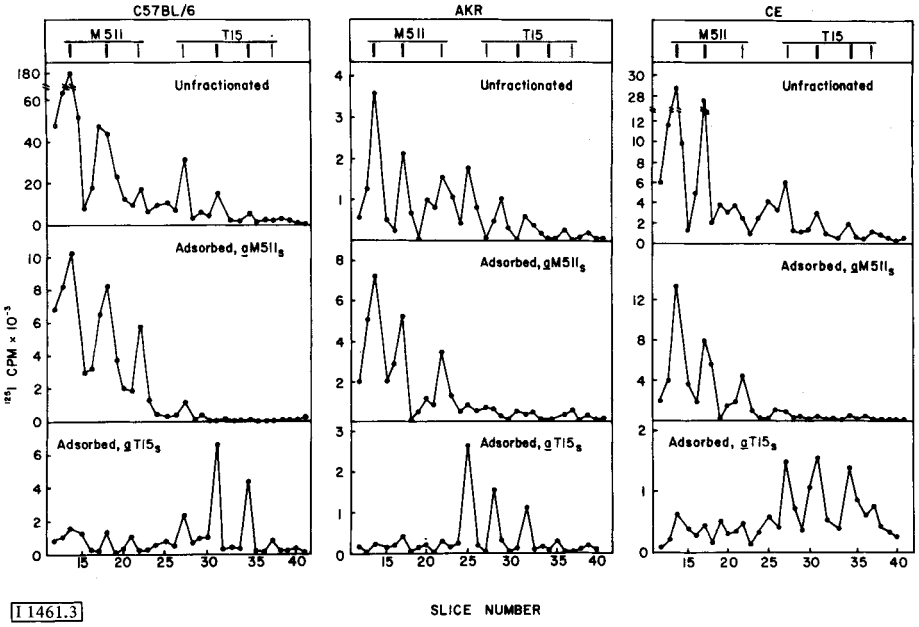


Figure 3. IEF patterns of L chains from C57BL/6, AKR, and CE ^{125}I-labeled anti-PC antibodies adsorbed with either anti-M511$_s$ or anti-T15$_s$. IEF of a portion of each sample was carried out in 0.3 x 12 cm polyacrylamide gel tubes in pH 5 to 9.5 ampholytes (see Scheme 1).

here, were obtained with the anti-PC antibodies from C58, CE and A mice. In BALB/c, fractionation with anti-M511$_s$ yielded little activity. These data indicate that immunoglobulins bearing T15 and M511 IDD contain L chains that are uniform and resemble those of T15 and M511, respectively.

4. Discussion

The present study presents a dissection of the anatomy of the B lymphocyte response to PC by examination of the composition of antibodies comprising the response. Nine different strains of mice were examined that differed at multiple genetic loci, especially the immunoglobulin H chain complex (allotype) locus. Quite surprisingly we find that not only is the response limited to relatively few species in each mouse strain, but it appears that each strain of mice contains an equivalent set of antibodies, and thus clonotypes, specific for PC. This strongly implies a conservation in mice (in *Mus musculus* ?) of genes encoding for antibodies to PC.

The basis for this interpretation is provided by analytical IEF of L chains separated from purified anti-PC antibodies and quantitation of immunoglobulins bearing certain ID determinants. The results themselves thus depend upon the discriminatory power of these two procedures. IEF has proven to be a powerful tool which clearly has the ability to distinguish between two nearly identical myeloma proteins of the same class [4, 17] and to identify and follow the progress of individual ID or clonotypes in a heterogeneous response even when the responding clones number in the hundreds to thousands [18–24]. The assay becomes limiting when comparing two polypeptides that cofocus yet differ in the kinds and distribution of charged, as well as uncharged, amino acids. However, in instances where this is known to occur, *e.g.* M511 and M167 L chains [4], the two polypeptides differ only slightly from one another, in this case by one amino acid in the first 21 [25]. In the present instance IEF was used as a first approximation of similarity of antibodies to PC and PC-binding myeloma proteins. Indeed, anti-PC antibodies in different strains contain L chains which have the same pI as T15 and M511/M167 L chains. Taken alone these results would at best be suggestive, but in conjunction with the ID fractionation studies they provide strong evidence that 511- and T15-like L chains of anti-PC antibodies are structurally quite similar to those of M511 and T15, respectively.

ID analyses were performed with antibodies specific for the binding regions of the myeloma proteins [10]. Binding-site regions were chosen primarily because the selective pressures exerted on binding (complementarity) regions and nonbinding site (framework) regions are surely different, and studies with antisera to such regions provide conflicting conclusions on antibody diversity [16, 26]. The anti-ID antibodies used here, in addition to being directed to the combining region, recognize a particular H-L combination. They are also noncross-reactive. Such antibodies should provide the most useful probes for defining structurally related antibodies. In fact, the antisera were able to readily distinguish between M511- and M167-like antibody in the anti-PC pools. Similarly, immunoglobulin(s) having an L chain like M603 was readily differentiated from M603.

Using a combination of these two procedures, ID and IEF analyses, two major species of antibodies were detected in each of nine different strains of mice. One of these corresponded to T15 and the other to M511. If further studies now in progress demonstrate that the M603-like L chain (L_{ii}), and the fourth L chain type, L_{iii}, are associated with immunoglobulins having single binding-site ID, then two additional antibody species are identified. Thus, the response to PC in each strain appears to be essentially equivalent. Structural studies of anti-PC antibody from A mice support these assertions. By ID and IEF analysis this antibody preparation consists of T15, M511, and M603-like antibodies. When the sequence of the A/J H chain was compared to the H chains of T15, M511 and M603, we found that both the framework and first complementarity region are identical in all of the proteins. Sequence analysis of the L chains through the first complimentarity region revealed three L chains, one similar to each of the three myeloma proteins, T15, M511 and M603.

Minor differences with the BALB/c myeloma proteins were evident but occurred primarily in framework regions.* Thus A/J and BALB/c, which differ genetically as much as any other two strains having different allotypes, express antibodies which are essentially identical.

This conservation of antibodies to PC is an unusual though not entirely unique finding for a set of immunoglobulins having a similar specificity. In the response to Group A streptococcal carbohydrate [27] and to p-azophenylarsonate [28], for example, strain-specific rather than species-specific idiotypy was observed. Similar findings have been reported by others for a variety of immune responses [26, 29, 30] suggesting that strain-specific idiotypy is the rule rather than the exception. However, interstrain ID cross-reactions have been demonstrated in both mice [31, 32] and rats [33] and, in some instances the anti-ID antisera recognized site-associated determinants. Structural correlates are unfortunately lacking. The data, nevertheless, raise the question of whether IDD that are confined to a strain (or a group of related strains) signify strain-specific antibody molecules or actually represent genetic differences for otherwise identical antibodies. Resolution of this latter point is particularly crucial since it argues for extensive conservation of antibodies throughout mouse strains but allows for some polymorphism within a common or equivalent set of genes. Such polymorphism may be minor (though giving rise to significant antigenic differences) and may be more likely to occur in framework residues. T15-like antibodies in mice are probably a specific example of this phenomenon. Preservation of the antibody throughout mouse strains has apparently occurred while allowing for the existence of minor structural differences between strains, *e.g.* the T15 ID [3] and K-PC8 L chain marker [34]. Whether this represents a unique observation cannot be determined at present. A closer examination of the anti-arsonate and anti-streptococcal responses as well as other antibody responses should be quite revealing.

The finding of an equivalent set of antibodies for PC in genetically dissimilar mice implies that genes encoding for these antibodies are likewise quite similar or, in some cases, identical. The most logical genetic explanation for these results is that the information for these sets of antibodies is contained in the germ line. A somatic mutational process would require simultaneous parallel mutations in each strain, presumably through very strong selective pressures of antigen. This seems unlikely. The gene insertion model proposed by some [35, 36] seems equally untenable. In the present system one would be forced to argue for parallel evolution of genes coding for both framework and complementarity regions. It seems more reasonable that a single germ line gene codes for each entire V_L and V_H and that these are preserved relatively unchanged in each strain of mice. The need for maintenance of these genes can readily be argued for since PC is a ubiquitous cell wall component in a variety of micro-organisms, *e.g. Streptococcus* sp., *Proteus* sp., *Aspergillus, Ascaris* [37], some of which are potential pathogens.

The report by Kluskens et al. [38] that BALB/c antibodies to PC (R36A) possess the single ID, T15, but are oligoclonal is perplexing. The oligoclonality of the response is based primarily on their finding that peptide maps, amino acid composition, and disc-electrophoretic patterns in urea gel of BALB/c L chains demonstrated heterogeneity. Although Kluskens et al. [38] may actually have induced a heterogeneous response entirely of the T15 idiotype, it seems more likely that their preparation was contaminated by the low molecular weight component, pI 5.7 ± 0.5 [4], which we find adheres nonspecifically to Sepharose columns and that we remove selectively with Cbz-glycine. No mention of removing this component was made by Kluskens and much of the heterogeneity in their L chain preparation could be accounted for by material focusing in the pH range 5.5 to 6.0.

However, in keeping with their findings [39] and those from other laboratories [11, 39], we find a low level of antibodies bearing other ID in BALB/c. Whether this reflects clonal dominance of the T15 clonotype in BALB/c or specific negative regulation has not been determined. Similar reasons may account for the poor representation of antibodies bearing M167 or M603 IDD. Alternatively, these clonotypes may be represented at very low frequencies in the B cell pool. Indeed, the ability to make isologous anti-ID antibody to the PC-binding myeloma proteins (M167 > M603 ⩾ M511 > T15) [40] is *inversely* related to the ability to produce anti-PC antibody possessing these ID, suggesting the presence of T15 and M511 clonotypes (and therefore tolerance to them) and the absence of M167 and M603 clonotypes (and susceptibility to immunization by them).

In conclusion, it must be kept in mind that we are examining the products of clonotypes predominating in the immune response and not the potential for a response that exists in the precursor B cell pool. The latter may contain additional clonotypes [11], determined by the germ line or generated anew, which are not stimulated by our immunization procedure with R36A. Other PC-containing antigens may stimulate these clones. Irregardless, a distinction must be made between the repertoire of precursor clonotypes and the actual responding clonotypes. This is especially important for those precursor B cells which are shown to develop somatically.

I am indebted to Margaret Kopchick for her excellent technical assistance and diligence in performing the IEF analyses.

Received June 29, 1976.

5. References

1 Claflin, J.L. and Davie, J.M., *J. Immunol.* 1974. *113*: 1678.

2 Claflin, J.L. and Davie, J.M., *J. Exp. Med.* 1974. *140*: 673.

3 Lieberman, R., Potter, M., Mushinski, E.B., Humphrey, W. and Rudikoff, S., *J. Exp. Med.* 1974. *139*: 983.

4 Claflin, J.L., Rudikoff, S., Potter, M. and Davie, J.M., *J. Exp. Med.* 1975. *141*: 608.

5 Claflin, J.L., Lieberman, R. and Davie, J.M., *J. Immunol.* 1974. *112*: 1747.

6 Greenwood, F.C., Hunter, W.M. and Glover, J.S., *Biochem. J.* 1963. *89*: 114.

7 Small, P.A. and Lamm, M.E., *Biochemistry* 1966. *5*: 259.

8 Wrigley, C.W., *J. Chromatogr.* 1968. *36*: 362.

9 Williamson, A.R., *Eur. J. Immunol.* 1971. *1*: 390.

* Rudikoff, S. and Claflin, J.L., Expression of equivalent clonotypes in BALB/c and A/J mice following immunization with phosphorylcholine. *J. Exp. Med.* 1976, in press.

10 Claflin, J.L. and Davie, J.M., *J. Immunol.* 1975. *114*: 70.

11 Gearhart, P.J., Sigal, N.H. and Klinman, N.R., *J. Exp. Med.* 1975. *141*: 56.

12 Rudikoff, S. and Potter, M., *Biochemistry* 1974. *13*: 4033.

13 Miller, F. and Metzger, H., *J. Biol. Chem.* 1965. *240*: 4740.

14 Williamson, A.R., Salaman, M.R. and Kreth, R.W., *Ann. NY Acad. Sci.* 1973. *209*: 210.

15 Kunkel, H.G., Agnello, V., Joslin, F.G., Winchester, R.J. and Capra, J.D., *J. Exp. Med.* 1973. *137*: 331.

16 Claflin, J.L. and Davie, J.M., *J. Exp. Med.* 1975. *141*: 1073.

17 Murgita, R.A. and Vas, S.I., *J. Immunol.* 1970. *104*: 514.

18 Askonas, B.A., Williamson, A.R. and Wright, B.E.G., *Proc. Nat. Acad. Sci. US* 1970. *67*: 1398.

19 Briles, D.E. and Davie, J.M., *J. Immunol. Methods* 1975. *8*: 363.

20 Cramer, M. and Braun, D.G., *J. Exp. Med.* 1974. *139*: 1513.

21 Eichmann, K., *Eur. J. Immunol.* 1974. *4*: 296.

22 Freedman, M.H., Pincus, J.H., Yeger, H., McKenny, J.A. and Mage, R.A., *Eur. J. Immunol.* 1974. *4*: 553.

23 Klinman, N.R. and Press, J.L., *J. Exp. Med.* 1975. *141*: 1133.

24 Kreth, H.W. and Williamson, A.R., *Eur. J. Immunol.* 1973. *3*: 141.

25 Apella, E. and Inman, J.K., in Reisfeld, R.A. and Mandy, W.T. (Eds.) *Contemporary Topics in Molecular Immunology*, Plenum Press, New York 1973, p. 51.

26 Weigert, M., Potter, M. and Sachs, D., *Immunogenetics* 1975. *1*: 511.

27 Eichmann, K., *Eur. J. Immunol.* 1972. *2*: 301.

28 Pawlak, L.L. and Nisonoff, A., *J. Exp. Med.* 1973. *137*: 855.

29 Eichmann, K., *Immunogenetics* 1975. *2*: 491.

30 Kindt, T.J., Thunberg, A.L., Mudgett, M. and Klapper, D.G., in Sercarz, E.E., Williamson, A.R. and Fox, C.F. (Eds.) *The Immune System: Genes, Receptors, Signals*, Academic Press, New York 1974, p. 69.

31 Briles, D.E. and Krause, R.M., *J. Immunol.* 1974. *113*: 522.

32 Kuettner, M.G., Wang, A. and Nisonoff, A., *J. Exp. Med.* 1972. *135*: 579.

33 Stankus, R.P. and Leslie, G.A., *Immunogenetics* 1976. *3*: 65.

34 Claflin, J.L., *Eur. J. Immunol.* 1976. *6*: 666.

35 Wu, T.T. and Kabat, E.A., *J. Exp. Med.* 1970. *132*: 211.

36 Capra, J.D. and Kindt, T.J., *Immunogenetics* 1976. *1*: 417.

37 Potter, M., *Physiol. Rev.* 1972. *52*: 692.

38 Kluskens, L., Lee, W. and Köhler, H., *Eur. J. Immunol.* 1975. *5*: 489.

39 Cosenza, H., Quintáns, J. and Lefkovits, I., *J. Immunol.* 1975. *5*: 343.

40 Sakato, N. and Eisen, H., *J. Exp. Med.* 1975. *141*: 1411.

I.A. Ramshaw°, P.A. Bretscher and C.R. Parish

Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra

Regulation of the immune response

I. Suppression of delayed-type hypersensitivity by T cells from mice expressing humoral immunity

The ability of horse red blood cell (HRBC)-specific T cells from mice expressing humoral immunity to suppress the induction of HRBC-specific delayed-type hypersensitivity (DTH) was investigated. The transfer of Ig-negative spleen cells, from mice injected 4 days previously with HRBC, completely suppressed the development of DTH in mice treated with cyclophosphamide and sensitized with HRBC. The suppressor cell was found to be lysed by treatment with anti-theta serum and complement. Furthermore, hemocyanin-specific immune T cells were able to suppress the DTH induced to HRBC, provided these two antigens were coupled together.

These studies suggest that T cells present under conditions where humoral immunity is induced can suppress DTH and that such cells play an important role in the regulation of the immune response.

1. Introduction

In many instances humoral and cell-mediated immunity (CMI) are mutually antagonistic [1]. For example, animals with humoral immunity are usually refractory to the establishment of delayed-type hypersensitivity (DTH) [2]. Two possible explanations for this refractory state are either that antibody or antigen-antibody complexes block the activated T cells which mediate DTH [3] or that T cells present in animals with humoral immunity actively suppress the induction of cell-mediated immunity. Recent reports [4, 5] have shown that T cells, induced under certain conditions, can actively suppress the development and expression of DTH.

A theoretical scheme, which has been recently devised [6], suggests that precursor cells for different types of immunity require different amounts of help to be induced. Thus, CMI requires the least, IgM an intermediate amount, and IgG the most. It is also postulated that T cells can inhibit the induction of other classes of immunity; in particular, larger amounts of T cell help than required for inductior

° Present address: Department of Microbiology and Immunology, Queens University, Kingston, Canada.

Correspondence: C.R. Parish, Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. 2601, Australia

Abbreviations: DTH: Delayed-type hypersensitivity **HRBC:** Horse red blood cells **SRBC:** Sheep red blood cells **CRBC:** Chicken red blood cells **CP:** Cyclophosphamide **HCY:** Hemocyanin **ABA:** Azobenzene arsonate **CMI:** Cell-mediated immunity **FIA:** Freund's incomplete adjuvant