CLONAL NATURE OF THE IMMUNE RESPONSE TO PHOSPHORYLCHOLINE

I. SPECIFICITY, CLASS, AND IDIOTYPE OF PHOSPHORYLCHOLINE-BINDING RECEPTORS ON LYMPHOID CELLS*

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The interaction of antigen with immunoglobulin-like receptors on immunocompetent lymphocytes is considered fundamental to the activation of these committed lymphocytes (1). Using a variety of techniques, independent laboratories have demonstrated the existence of such receptor-bearing cells and their predominantly bone marrow (B)-lymphocyte nature. Selective depletion or enrichment of lymphoid cell function by passage through antigen-coated immunoadsorbents (2–4), by treatment with heavily labeled radioactive antigen (5), or after specific fluorescent labeling and separation in a photoactivated cell sorter (6) has demonstrated indirectly the immunocompetence of these cells. More direct evidence for the presence of lymphocyte receptors has been obtained from autoradiographic studies of specific binding of radioactive antigen to the surface of lymphocytes (4, 7–9). In these studies, the receptors were found to possess heavy- and light-chain determinants (10), and the high degree of hapten specificity (4, 9, 11) classically attributed to circulating antibody. However, neither the immunocompetence of these antigen-binding cells nor their relationship to precursor cells has been proven.

A corollary theme and one central to clonal selection theories of antibody formation is that the receptors on a given clone of immunocompetent cells possess essentially the same structural features, at least in the region of the antibody-combining site, as the antibody molecules to be secreted by the descendents of this clone of precursor cells (1). Indirect evidence in support of this hypothesis has been provided by recent studies which have utilized antisera directed to variable region, if not binding site, antigenic determinants. Passive transfer of such specific anti-idiotypic antiserum into unimmunized mice renders them incompetent to produce antibody bearing that idiotype upon immunization (12, 13). This suggests the existence, before immunization, of a population of committed lymphocytes with receptors bearing the same idiotypic determinants as circulating antibody.

We have pursued directly the relationship between cell surface receptor and secreted antibody molecules in the immune response to phosphorylcholine

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The present investigation describes the nature and specificity of the receptor on PC-specific antigen-binding lymphocytes (ABC) in unimmunized and immunized mice. Our findings demonstrate that the receptors on these ABC are IgM immunoglobulins which have a high degree of hapten specificity and, in the case of one inbred strain, BALB/c, possess the idiotypic specificity of a PC-binding myeloma, HOPC 8.

**Materials and Methods**

**Animals and Plasmacytomas.**—Female BALB/cJ, CBA/J, A/J, AKR/J, and (CAJ)J mice (6–8 wk old) were obtained from Jackson Laboratories, Bar Harbor, Maine. The 2,4-dinitrophenyl (DNP)-binding mouse plasmacytoma, MOPC 315 (α, λ) and the PC-binding plasmacytomas, HOPC 8, TEPC 15, M/PC 603, MOPC 511, and MOPC 167 (all α, κ) were given to us by Dr. M. Potter, National Cancer Institute, Bethesda, Md. These myeloma tumors were maintained by intraperitoneal (i.p.) or subcutaneous (s.c.) transfer in BALB/cJ mice.

**Reagents.**—PC was purchased from Calbiochem, San Diego, Calif.; L-α-glycerophosphorylcholine (GPC), choline (C), and α-DNP-L-lysine were obtained from Sigma Chemical Co., St. Louis, Mo. The calcium and cadmium ions in PC and GPC, respectively, were precipitated with phosphate before use. p-Nitrophenylphosphorylcholine, a gift from Dr. B. Chesebro, Rocky Mountain Laboratory, NIAID, Hamilton, Mont., was reduced to the amine and diazotized with NaNO₂ according to the procedure of Chesebro and Metzger (14). The resulting p-diazonium phenylphosphorylcholine (DPFC) solution was frozen and stored at −15°C until used.

**Hapten-Carrier and Erythrocyte Conjugates.**—Phosphorylcholine-protein conjugates were produced by reacting an appropriate concentration of DPPC with bovine serum albumin (BSA) (Sigma) and with keyhole limpet hemocyanin (KLH) (Calbiochem) in borate-saline buffer (0.035 M Na₂B₄O₇ and 0.08 M NaCl), pH 9.0, for 18 h at 4°C. At the end of the reaction time the preparations were dialyzed successively against 0.5 M NaCl and against PBS, pH 7.2. The degree of coupling was 21 mol/mol for PC-BSA and 19 mol/100,000 daltons for PC-KLH. DNPᵢ-KLH and DNP₂ᵢ-BSA were prepared as previously described (4) by reacting 1-fluoro-2,4-dinitrobenzene with KLH. PC sheep red blood cells (PC-SRBC) optimal for plaque assay (15) of either immune spleen cells or myeloma cells were obtained as follows: 3.7 μmol DPPC in 0.1 ml were mixed with 3 ml of a 25% suspension of SRBC in borate-saline buffer. After 30 min at room temperature the PC-SRBC were washed once in the reaction buffer and three times in Tris-buffered saline. Trinitrophenyl-SRBC (TNP-SRBC) were prepared by the method of Rittenberg and Pratt (16).

**Antisera.**—Class-specific antisera to mouse heavy chains were produced in goats by multiple s.c. injections of purified mouse myeloma proteins in complete Freund’s adjuvant (Difco Laboratories, Detroit, Mich.). These proteins, RPC 24 (γ₂₅) and RPB 23 (γ₁), were gifts from Dr. Alexander Lawton, University of Alabama, Birmingham. A mixture of these antisera was absorbed in the cold with washed MOPC 104 (μ, λ₁) and MOPC 460 (α, κ) tumor cells to remove anti-histocompatible, anti-μ and anti-κ activity. Purified anti-μ (MOPC 104), a gift from Dr. Lawton, was prepared from agarose immunoadsorbant columns to which purified mouse immunoglobulins had been coupled. The anti-μ antiserum was fluo-

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1 *Abbreviations used in this paper: ABC, antigen-binding lymphocytes; BSA, bovine serum albumin; C, choline; DNP, 2,4-dinitrophenyl; DPPC, p-diazonium phenylphosphorylcholine; F₁-anti-μ, fluoresceinated anti-μ; GPC, L-α-glycerophosphorylcholine; KLH, keyhole limpet hemocyanin; MEM, minimal essential medium; PC, phosphorylcholine; PFC, antibody-producing cells; SRBC, sheep red blood cells; TNP, trinitrophenyl; Tx-BM, thymectomized, bone marrow-reconstituted mice.*
resuspended by a dialysis method (17) using fluorescein isothiocyanate (Sigma). The specificity of this reagent for \( \mu \)-determinants was shown by the fact that it gave cytoplasmic fluorescence with MOPC 104, but not with MOPC 315 (\( \alpha, \lambda \)), HOPC 8 (\( \alpha, \kappa \)), MOPC 70 (\( \gamma_{1}, \kappa \)), MOPC 195 (\( \gamma_{2b}, \kappa \)), or LPC-1 (\( \gamma_{2b}, \kappa \)) tumor cells.

Antisera specific for the idiotypic determinants of the PC-binding myeloma proteins were prepared by immunizing AL and A/He mice with purified myeloma immunoglobulins (18). Antiallotype antibody was removed by immunoadsorption on Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) coupled with normal BALB/c serum proteins. These antisera reacted specifically in gel diffusion and hemagglutination assays, and in the case of HOPC 8, MOPC 167, and MOPC 603 the hemagglutination reaction between SRBC-myeloma protein and specific antisera was inhibitable with \( 10^{-8} \) M PC but not with an unrelated ligand, DNP-lysine. Anti-MOPC 315 antibody was prepared in BALB/c mice according to the procedure of Sirisinha and Eisen (19).

**Thymectomy.**—Adult, female AKR mice (5–6 wk of age) were surgically thymectomized. 4 wk later, the mice received 750-R irradiation followed by 5–10 \( \times \) 10\(^{8} \) syngeneic bone marrow cells administered i.v. After an additional 4 wk, at which time they were immunized, the thymectomized, bone marrow-reconstituted (Tx-BM) mice had undetectable levels of \( \theta \)-bearing lymphocytes and normal or slightly elevated frequencies of immunoglobulin-bearing cells. Histologic examination of lymphoid organs showed sparse numbers of lymphocytes in thymus-dependent areas and normally populated B zones.

**Immunization and Preparation of Cell Suspensions.**—Mice were immunized i.p. with single or multiple injections of 1–1000 \( \mu \)g PC-KLH or with 10\(^{3} \) heat-killed (56°C, 30 min) *Diplococcus pneumoniae* strain R36A, an organism which contains phosphorylcholine as a cell wall component (20, 21). PC-KLH was administered s.c. in the inguinal region in complete Freund’s adjuvant (Difco) or i.p. with 10\(^{3} \) *Bordetella pertussis* organisms (Eli Lilly and Co., Indianapolis, Ind.). Occasionally mice were immunized i.p. with 100–500 \( \mu \)g DNP-KLH suspended with the pertussis vaccine or Freund’s complete adjuvant. At intervals after immunization spleen or lymph nodes were harvested and single cell suspensions were prepared in minimal essential medium (MEM) (Microbiological Associates Inc., Bethesda, Md.). For antigen-binding assays, erythrocytes were lysed by treatment with NH\(_{4}\)Cl (22).

**Detection of Antigen-Binding Cells and Characterization of Their Receptors.**—\( ^{151} \)I-labeled PC-BSA and DNP-BSA were prepared by the chloramine T method (23). Different preparations of \( ^{35} \)I-PC-BSA were used in these experiments and ranged in sp act from 11 to 25 \( \mu \)Ci/\( \mu \)g. The single preparation of \( ^{125} \)I-DNP-BSA had a sp act of 28 \( \mu \)Ci/\( \mu \)g. ABC with receptors for PC or for DNP were detected as previously described (4). Briefly, 50–200 ng of radioliodinated antigen in 5–10 \( \mu \)l was added to 0.05–0.2 ml of cell suspension (\( \sim150 \times 10^{6} \) cells/ml) in MEM containing 10% fetal calf serum (Reheis Chemical Co., Chicago, Ill.) and 1 mg/ml sodium azide. In some experiments (detailed in Results) 5–10 \( \mu \)l of inhibitor (anti-serum or hapten) was also added. After 30-min incubation on ice the cells were suspended in 1 ml MEM and layered over 3 ml fetal calf serum in 17 X 10-mm polypropylene tubes. The cells were sedimented by centrifugation at 250 g for 10 min. This wash through fetal calf serum was repeated three times. After determining the radioactivity of the final cell pellet in a \( \gamma \)-ray spectrometer, the cells were suspended in medium and smeared on fetal calf serum-subbed slides. The slides were then air-dried, fixed in 1% glutaraldehyde, and dipped in NTB-2 emulsion (Eastman Kodak, Rochester, N. Y.). The exposure time was generally 5–10 days in PC-binding assays and 1–3 days in DNP-binding assays. ABC were enumerated in methyl green-pyronin stained autoradiographs as those lymphoid cells having six or more grains over or touching their surface.

Certain experiments combined radioautography and immunofluorescence in a double-label procedure described in detail elsewhere (24). Cells were incubated with \( ^{151} \)I-antigen and sedimented twice through fetal calf serum. They were then suspended in medium plus azide containing fluoresceinilated anti-\( \mu \) (Fl-anti-\( \mu \)). After 30 min incubation on ice the cells were...
washed two more times in fetal calf serum, fixed in methanol:acetic acid:water (89:1:10) and processed for autoradiography but not stained. ABC were first identified by dark-field microscopy and then examined for coincident fluorescence.

**Plaque Assay Procedure.**—Anti-PC-specific antibody-producing cells (PFC) were detected by a hemolytic plaque assay using PC-SRBC as the indicator cells. Anti-DNP PFC were detected using TNP-SRBC. IgM PFC were measured in direct plating assays and IgG PFC in indirect assays using a polyvalent goat antimouse immunoglobulin antiserum. Inhibition of PFC by the incorporation of different concentrations (10^{-2}-10^{-7} M) of specific, soluble haptens in the agar served as a means of measuring the relative affinity of PFC antibodies (25, 26).

**RESULTS**

Lymphoid organs of nonimmunized mice contained exceedingly low numbers of small lymphocytes which bound [125I]PC-BSA to their surface. The frequency of ABC in spleens and popliteal and mesenteric lymph nodes ranged from 0.3 to 5 in 10^5 cells but in the thymus this frequency was less than 1 in 10^6 cells. After immunization with pneumococci the frequency of PC-specific ABC increased and a larger percentage of the cells with silver grains on their surface were medium to large lymphocytes having pronounced pyroninophilia. Plasma cells comprised a very small minority of the total ABC in immune mice. In immunized animals some macrophages were encountered which bound [125I]PC-BSA but these could be differentiated both morphologically and by the fact that they characteristically had greater than 100 surface-associated grains.

**Kinetics of ABC After Immunisation.**—The frequency of PC-specific ABC and IgM PFC in BALB/c, A, and CBA strain mice found in the spleen following immunization with one or more injections of 10^6 pneumococci is shown in Fig. 1. The PFC response in these mice was entirely of the IgM class; no IgG or IgA PFC were detected. Though minor differences existed among the three strains, the dynamics of the anti-PC responses were quite similar. Before immunization, only rare lymphoid cells bound [125I]PC-BSA. After immunization, this frequency increased 100-fold, peaked on day 5, then declined to near preimmune levels by days 8-10. After a second injection of antigen the frequency of ABC again rose and fell but in a pattern similar to that observed in the primary response. The PFC responses to PC, also shown in Fig. 1, closely paralleled the ABC both in frequency and in their kinetics.

**Thymus Independence of the Anti-PC Response.**—Normal and Tx-BM female AKR mice were immunized with pneumococcal organisms and at intervals thereafter were tested for frequencies of the PC-specific ABC and PFC. As demonstrated in Table I, the kinetics of the ABC and the PFC responses in both normal and thymus-deprived mice were very similar, indicating a lack of requirement for T cells in the IgM anti-PC response. A similar degree of thymus independence was noted in the proliferation of ABC and PFC in the IgM, but not the IgG, response to DNP. The IgG PFC response to this hapten has been shown previously to be strongly T-dependent (27).

**Specificity of Antigen-Binding by PC-ABC.**—BALB/c mice were immunized with pneumococci and the number of cells binding [125I]PC-BSA in the spleen...
Fig. 1. Frequency of PC-specific ABC and PFC in BALB/c, A, and CBA mice after immunization with pneumococci. Mice were immunized i.p. with 10⁶ pneumococci (indicated by arrows) and tested at intervals for the numbers of splenic ABC and PFC. The means ± SE for three separate experiments are shown.
TABLE I

Thymus Independence of the Anti-PC Response

<table>
<thead>
<tr>
<th>Time after</th>
<th>Normal AKR</th>
<th>Tx-BM AKR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABC/10^6 lymphocytes</td>
<td>Direct PFC/10^6</td>
</tr>
<tr>
<td>Primary, day 2</td>
<td>9 ± 5</td>
<td>68 ± 16</td>
</tr>
<tr>
<td>Primary, day 3</td>
<td>--</td>
<td>113 ± 65</td>
</tr>
<tr>
<td>Primary, day 5</td>
<td>43 ± 16</td>
<td>230 ± 31</td>
</tr>
<tr>
<td>Secondary, day 6</td>
<td>28 ± 4</td>
<td>157 ± 25</td>
</tr>
<tr>
<td>DNP-response*</td>
<td>43 ± 23</td>
<td>--</td>
</tr>
<tr>
<td>Nonimmune</td>
<td>1,050 ± 170</td>
<td>54 ± 12</td>
</tr>
</tbody>
</table>

* Normal and Tx-BM AKR mice were immunized on day 0 and 11 with 10^8 pneumococci (PC-response) or with 100 μg DNP-KLH in CFA on day 0 and 100 μg DNP-KLH in saline on day 21 (DNP-response). At intervals, spleens were assayed for specific ABC and PFC.

was determined 5 days later. Cells binding [125I]DNP-BSA in these same spleens were also studied. The data in Table II demonstrate that the interaction between [125I]PC-BSA and splenic lymphoid cells was hapten-specific. PC at 8 × 10^{-3} M and PC-ovalbumin at a PC concentration of 8.2 × 10^{-8} M inhibited PC-BSA binding by 70-97% but did not inhibit DNP-BSA binding appreciably. Concentrations of GPC and C up to 10^{-2} M did not inhibit antigen binding by PC-ABC without having a concurrent adverse effect on [125I]DNP-BSA binding by DNP-ABC. No significant inhibition of PC-BSA binding by an unrelated hapten (DNP-lysine) by itself or coupled to the carrier molecule (BSA) was achieved.

Immunoglobulin Class of Receptors on PC-ABC.—The immunoglobulin nature of the receptor on PC-ABC was examined in preliminary experiments through inhibition of binding of [125I]PC-BSA by anti-immunoglobulin sera. The data from such studies on ABC obtained 4 days after primary and secondary immunization of BALB/c are shown in Table III and suggest that the receptors on ABC in immune mice are predominantly of the IgM class. Antibodies specific for μ-determinants at a concentration as low as 25 μg/ml caused marked diminution in the frequency of ABC. By contrast, much less inhibition of antigen binding was achieved with antisera directed toward other heavy-chain classes, even when they were used at 40-100-fold higher concentrations. To more directly study the Ig class of the receptor on ABC, a double-label procedure was employed (24) in which spleen cells were incubated sequentially with [125I]PC-BSA and F1-anti-μ. The latter reagent stained 41.7 ± 3% of
TABLE II
Specificity of Antigen-Binding By ABC for $[^{3}H]$PC-BSA

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>ABC*</th>
<th>Inhibition†</th>
<th>Total cells counted</th>
<th>$\text{ABC/10}^a$ Lymphocytes</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PC</td>
<td>100,000</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC, $8 \times 10^{-2}$ M</td>
<td>210,000</td>
<td>9</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC, $8 \times 10^{-4}$</td>
<td>185,000</td>
<td>27</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC-OVA, $8.2 \times 10^{-3}$ M</td>
<td>167,000</td>
<td>1</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNP-lysine, $5 \times 10^{-2}$ M</td>
<td>155,000</td>
<td>30</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNP-BSA, $5 \times 10^{-4}$ M</td>
<td>163,000</td>
<td>29</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNP</td>
<td>27,000</td>
<td>166</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNP-lysine, $5 \times 10^{-3}$ M</td>
<td>25,000</td>
<td>69</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC, $8 \times 10^{-2}$ M</td>
<td>20,000</td>
<td>152</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PC</td>
<td>125,000</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC, $8 \times 10^{-4}$ M</td>
<td>199,000</td>
<td>12</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GPC, $5 \times 10^{-4}$ M</td>
<td>266,000</td>
<td>41</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C, $5 \times 10^{-4}$ M</td>
<td>120,000</td>
<td>43</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNP</td>
<td>66,000</td>
<td>145</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC, $8 \times 10^{-3}$ M</td>
<td>37,000</td>
<td>152</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GPC, $5 \times 10^{-4}$ M</td>
<td>45,000</td>
<td>140</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C, $5 \times 10^{-4}$ M</td>
<td>43,000</td>
<td>133</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

* Spleen cells from BALB/c mice immunized 5 days previously with $10^8$ pneumococci were tested with $[^{3}H]$PC-BSA and with $[^{3}H]$DNP-BSA. Immunization with pneumococci caused no significant increase in either DNP-specific ABC or PFC.
† The inhibitors were added 20 min before and during incubation with the radioactive antigen. The molarity refers to the final concentration of the hapten, PC, or DNP.

TABLE III
Effect of Anti-Immunoglobulin Antiserum on Binding of $[^{3}H]$PC-BSA by ABC

<table>
<thead>
<tr>
<th>Exp. no.*</th>
<th>Antiserum†</th>
<th>$\text{ABC/10}^a$ Lymphocytes</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NGS</td>
<td>47</td>
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<tr>
<td></td>
<td>Anti-$\mu$, 2,500 $\mu$g/ml</td>
<td>3</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Anti-$\gamma_1$, 1,000 $\mu$g/ml</td>
<td>41</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Anti-$\gamma_1$, 1,000 $\mu$g/ml</td>
<td>52</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>NGS</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-$\mu$, 250 $\mu$g/ml</td>
<td>7</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Anti-$\mu$, 25 $\mu$g/ml</td>
<td>16</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Anti-$\alpha$, 600 $\mu$g/ml</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Anti-$\gamma_1$ &amp; $\gamma_2$, 1,000 $\mu$g/ml</td>
<td>50</td>
<td>22</td>
</tr>
</tbody>
</table>

* Spleens were obtained either 4 days (Exp. 1) or 14 days (Exp. 2) after immunization of BALB/c mice with pneumococci on days 0 and 10.
† Normal goat serum or goat antihuman chain antisera were added 20 min before and during exposure to $[^{3}H]$PC-BSA.
splenic lymphocytes and less than 0.1% of thymus cells from normal or immunized mice. Among ABC staining with F1-anti-\(\mu\), (Fig. 2) approximately 85% displayed a patchy or diffuse fluorescence indicating a random distribution of Ig on the cell surface. The remaining 15% showed only polar fluorescence suggesting that modulation of surface Ig had occurred.

![Image](image_url)

**Fig. 2.** Coincident binding of \[^{125}\text{I}\]PC-BSA and F1-anti-\(\mu\) by BALB/c splenic lymphoid cells. Quadrants A and B show the silver grain distribution over two cells binding \[^{125}\text{I}\]PC-BSA (dark-field microscopy) and quadrants a and b show the same cells stained with F1-anti-\(\mu\) (fluorescent microscopy).

Table IV summarizes studies on the Ig class of the receptor on PC-binding cells in different inbred strains of mice. As can be seen, essentially all of the PC-specific ABC in nonimmune and immune mice, regardless of strain, possessed \(\mu\)-determinants. By comparison, the percent of DNP-binding ABC in DNP-KLH immune mice that stained with F1-anti-\(\mu\) decreased from 86% on day 3 of the primary response to 23% in hyperimmunized mice. Thus, in contrast to the usual, time-dependent decline in proportion of IgM-bearing ABC
### TABLE IV

**Immunoglobulin Class and Idiotype of the Receptor on PC-ABC**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time after Immunization</th>
<th>% μ-bearing* ABC</th>
<th>% Inhibition of ABC by anti-idiotypic antisera to:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HOPE 8</td>
</tr>
<tr>
<td>BALB/c</td>
<td>(PC Response)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 0§</td>
<td>&gt;94</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Primary, day 3</td>
<td>97</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Primary, day 6</td>
<td>&gt;96</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Secondary, day 7</td>
<td>98</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Hyperimmune</td>
<td>&gt;96</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>Primary, day 3</td>
<td>&gt;96</td>
</tr>
<tr>
<td></td>
<td>Primary, day 7</td>
<td>&gt;97</td>
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<td></td>
<td>Secondary, day 6</td>
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<td></td>
<td>Hyperimmune</td>
<td>&gt;98</td>
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<tr>
<td></td>
<td>CBA</td>
<td>Primary, day 3</td>
<td>&gt;92</td>
</tr>
<tr>
<td></td>
<td>Primary, day 8</td>
<td>&gt;99</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Secondary, day 7</td>
<td>&gt;95</td>
<td>—</td>
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<tr>
<td></td>
<td>Hyperimmune</td>
<td>98</td>
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<td>BALB/c</td>
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<td>Day 0§</td>
<td>89</td>
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<tr>
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<td>Primary, day 3</td>
<td>86</td>
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</tr>
<tr>
<td></td>
<td>Primary, day 9</td>
<td>69</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Secondary, day 8</td>
<td>23</td>
<td>—</td>
</tr>
</tbody>
</table>

* Per cent of PC- or DNP-specific ABC that bound F1-anti-μ.
† Antiserum diluted to a final concentration of 1:10.
§ Spleens were tested after one (day 0), two (days 0 and 9–10), or multiple (days 0, 9–10, and 20–41) injections of 10⁶ pneumococci (PC-response) or after one (day 0) or two (days 0 and 10) injections of 500 μg DNP-KLH. i.p.
|| Indicates less than 15% inhibition.

In the anti-DNP response, the anti-PC response exhibits a striking lack of change in receptor class on specific ABC. The persistence of IgM receptors on PC-ABC is reflected in the mature PFC by an absence of IgG anti-PC antibody.

*Idiotype Specificity of the Receptor on PC-ABC.*—It has been reported (12, 13) that passive transfer of anti-idiotypic antisera can successfully prevent the subsequent appearance in immunized mice of antibody bearing that idiotype, suggesting that specific precursor cells have receptors with the same idiotype as secreted antibody. In the present study, the presence of specific immunoglobulin receptors for PC has been shown and further experiments were conducted to determine the idiotypes of these receptors. To spleen cells suspended in 50 μl of medium containing azide was added 5–10 μl of anti-idiotypic antiserum. After 20 min, [³⁵S]PC-BSA was added and the cell suspension was
processed for autoradiography as described in Materials and Methods. The results of experiments on different inbred mouse strains are shown in Table IV. Among the inbred strains tested, only the ABC in BALB/c were blocked from binding \(^{32}P\)PC-BSA by anti-idiotypic antisera. Moreover, of the antisera used, anti-HOPC 8, but not anti-M\(^{2}\)PC 603 or anti-MOPC 167, suppressed the frequency of ABC in these mice. The specificity of inhibition by anti-HOPC 8 was shown by the fact that the frequency of DNP-binding ABC in the pneumococci-immune BALB/c mice was not diminished by pretreatment with the antisera. None of the antisera significantly blocked antigen-binding by A or CBA mice.

To test for the possible appearance of new clones or for the outgrowth of clones represented to a minor degree at a particular time, the receptors on PC-ABC in BALB/c were examined for idiotypic determinants at different times during the anti-PC immune response. As indicated in Table IV, when tested at various times after immunization with pneumococci, only anti-HOPC 8 decreased the frequency of ABC, and this inhibition occurred about equally at each time point. Thus, the receptors on PC-ABC in BALB/c may possess only a single idiotype.

**Binding Specificity of Anti-PC Antibodies.**—Since the receptors on BALB/c PC-ABC possessed characteristics identified with HOPC 8 protein, experiments were carried out to determine if the antibody secreted in the BALB/c anti-PC response displayed a binding specificity similar to that of HOPC 8. BALB/c immune spleen cells and each of the PC-binding myeloma tumor cells were plaqued against PC-SRBC and a comparison was made of the quantity of PC, GPC, and C giving 50% inhibition of plaque formation. As shown in Table V both HOPC 8 and M\(^{2}\)PC 603 bound PC > GPC > C while MOPC 167 and MOPC 511 had different binding patterns. However, the former two myelomas could be distinguished by the fact that HOPC 8 had a higher relative specificity for PC than did M\(^{2}\)PC 603. Examination of the inhibition profiles obtained with BALB/c immune cells reveals that the secreted anti-PC antibody shows the same binding order for PC, GPC, and C, and more importantly the same relative affinity for these choline analogues as does HOPC 8. Moreover, the anti-PC antibody produced in BALB/c possesses the idiotypic specificities of HOPC 8 (28, 29). Thus, a direct link clearly exists between the receptors on specific ABC and secreted antibody in the anti-PC response in BALB/c mice.

**DISCUSSION**

A considerable body of evidence has accumulated which supports the existence and immunologic relevance of lymphocytes bearing surface-associated immunoglobul-
RECEPTORS ON PC ANTIGEN-BINDING CELLS

TABLE V

Binding Characteristics of BALB/c Anti-Phosphorylcholine Antibodies and of Phosphorylcholine-Binding Myeloma Proteins

<table>
<thead>
<tr>
<th>Cell population</th>
<th>PC</th>
<th>GPC</th>
<th>C</th>
<th>Relative specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I_50</td>
<td>I_50</td>
<td>I_50</td>
<td></td>
</tr>
<tr>
<td>BALB/c§</td>
<td>4.5</td>
<td>3.65</td>
<td>1.8</td>
<td>8</td>
</tr>
<tr>
<td>HOPC 8</td>
<td>5.4</td>
<td>4.4</td>
<td>2.55</td>
<td>10</td>
</tr>
<tr>
<td>M*PC 603</td>
<td>5.45</td>
<td>4.6</td>
<td>1.7</td>
<td>7.1</td>
</tr>
<tr>
<td>MOPC 167</td>
<td>4.5</td>
<td>4.85</td>
<td>3.9</td>
<td>0.5</td>
</tr>
<tr>
<td>MOPC 511</td>
<td>4.1</td>
<td>4.6</td>
<td>4.3</td>
<td>0.32</td>
</tr>
</tbody>
</table>

* I_50 = -log_{10} molar concentration of PC, GPC, and C required to give 50% inhibition of plaque formation.
† Relative specificity = (molarity of GPC or C at I_50)/(molarity of PC at I_50).
§ 4 day primary.

lin-like receptors, though the direct relationship of these cells to immunocompetence has not been demonstrated. It is clear that, even before immunization, rare lymphocytes bear surface immunoglobulins with specificity for antigen similar to that of serum antibody, and that upon immunization, the specific cell population increases. In addition, several studies have shown an association between the immunocompetence of a cell population and the presence of lymphocytes with the particular surface receptors. These studies have used selective depletion or enrichment of antigen-specific lymphocytes by the adsorption of these cells to insoluble antigens (2-4), selective killing by radiolabeled antigens (5), or, more recently, by use of fluorescein-tagged antigens and photosensitive cell sorters (6). The association between ABC and the immunocompetence of the cell population as well as the finding that most of such cells bear easily detectable immunoglobulin have supported the view that ABC are primarily the precursors of antibody-secreting cells, and that the lymphocyte receptor should be identical in its antigen-binding capacity to the immunoglobulin secreted by the mature progeny. However, the evidence as to the identity of ABC with precursors of antibody-secreting cells must still be considered as circumstantial. Indeed, several studies have failed to correlate immunocompetence with ABC (30, 31). This is probably due to the imperfect methods of depletion or purification of such cells and the large numbers of different clones of cells which must exist for most antigens.

This latter potential objection was removed with the report of Cosenza and Köhler (13) that the immune response of BALB/c mice to PC appeared to be pauciclonal in nature. They determined that the in vitro immune response to PC could be inhibited completely by pretreatment of nonimmune mouse spleen cells with anti-idiotypic antibody prepared against TEPC 15, an anti-PC-producing BALB/c plasmacytoma. In addition, the antibody of the majority of antibody-secreting cells in unsuppressed immunized BALB/c mice possessed this idiotypic determinant (13, 28). We have extended these observations to examine in part the relationships between ABC and PFC in a study of the immune response to PC in inbred strains of mice. For these purposes, the response was measured with a PC derivative, p-phenylphosphorylcho-
line, before or after immunization with \textit{D. pneumoniae} strain R36A, an organism known to contain PC as a cell wall component \cite{20, 21}.

In the present study, the nature of the receptors on ABC in unimmunized and immunized mice which bind $^{[35]}$PC-BSA was examined. In unimmunized mice of each strain tested these ABC were present in extremely low frequencies in lymph nodes and spleens ($\sim 1/10^6$ lymphocytes) and were essentially undetectable in the thymus. This is in marked contrast to ABC which bind other antigens \cite{32}, including haptens \cite{4}, which are normally present in much higher frequencies. The PC-ABC increase after immunization and have kinetics very similar to those of PC-specific PFC. The receptors on these ABC are hapten-specific and are exclusively of the IgM class.

There are several distinctive features in the immune response to PC. The first is the generally weak and transient increase in frequency in ABC upon even repeated immunizations. This fact is reflected in the absence of significant "memory" in secondary responses seen in this instance as well as in the response to other thymus independent antigens \cite{33}. In contrast to this finding is the extraordinarily efficient conversion of precursor cells (i.e., ABC) to mature antibody-secreting cells upon immunization. In fact, the frequencies and kinetics of ABC and PFC are so similar that one wonders whether some ABC may not be PFC. In the response to other antigens examined the distinction is not important since the frequency of ABC always greatly exceeds that of PFC. This is clearly not the case here. While some investigators have shown the presence of membrane-associated antigen-binding capacity on antibody-secreting cells \cite{34, 35}, others have not \cite{36}. However, the lymphocytic morphology of ABC is clearly at variance with the generally plasmocytic morphology seen for IgM PFC \cite{37}. Therefore, we feel confident that the ABC which we measure are indeed precursors and not PFC. This conclusion is supported by the recent findings of Wernet et al. \cite{38} who found that a percentage of small lymphocytes in individuals with macroglobulinemia carried idiotypic determinants similar to those of the circulating paraprotein.

In BALB/c mice, idiotypic antiserum to HOPC 8, one of a number of PC-binding myelomas which have a common idiootype \cite{39}, clearly inhibited the binding of $^{[35]}$PC-BSA by ABC. Other anti-idiotypic antisera did not inhibit binding. Moreover, the receptors on the ABC in BALB/c display the same relative specificity for PC and two of its analogues, GPC and C, as do PC-binding myelomas with the same idiotype as HOPC 8 \cite{40}. These data strongly indicate that the receptors on BALB/c PC-ABC have idiotypic determinants and specificities either identical to or cross-reactive with those of HOPC 8 and indicate that ABC are indeed immunologically relevant. The fact that cell receptors share with the serum immunoglobulin characteristics of class, specificity, and idiotype (along with the studies of others on the importance of precursor cells which bear the idiotypic determinants), make it very likely that ABC are the immunologically relevant precursor cells.

The fact that other idiotypes on ABC were not detected in BALB/c and...
that anti-HOPC 8 almost completely reduced binding of [125I]PC-BSA to the same degree at any time after immunization suggest that the serologic response to PC may be highly restricted or even monoclonal. Moreover, the antibody would be expected to have the same binding characteristics as HOPC 8. Such a prediction is supported by the data in Table V and the findings of others (28, 29, footnote 2).

A peculiar feature of the immune response to PC noted in the present work was the striking lack of change in the Ig class of the receptor on ABC with time after immunization. Thus, PC-ABC in unimmunized and in singly or multiply immunized mice of each strain continued to fluoresce with F1-anti-\(\mu\). These observations are consistent with the findings that the induced anti-PC response, even after repeated immunizations, is almost exclusively of the IgM class (28, 29). Moreover, as demonstrated here, the immune response to PC is thymus independent. Similar observations have been made in the IgM but not the IgG immune response to hapten-protein conjugates (27), to H-2 linked immune responses (41), and in the immune response to polysaccharides such as SSS-III (33), endotoxin (42), and levan (43). Our inability to stimulate an IgG anti-PC response is provocative, but may be explained by a lack of helper T cells such as occurs with DNP-p-GL (44) and in H-2 linked responses (41).

Previous studies from this (45) and other laboratories (32, 46) have strongly emphasized the immunologic relevance of ABC as progenitors of antibody-secreting cells. The receptors on ABC possess the same degree of specificity (11, 45-47), change in immunoglobulin class (48, 49), and changes in avidity during the immune response (25) observed for secreted antibodies. The present findings extend this correlation to the possession of an idiotypic on the receptor. These data strongly favor the original idea (1) that receptors on precursor cells are essentially immunoglobulin molecules and that the interaction of specific antigen with these receptors leads to cell activation, proliferation, and/or differentiation into progeny which secrete antibody molecules identical to those of the receptor on the precursor cells. Final proof of the precursor cell nature of ABC would require direct visualization of the conversion of an ABC to an antibody-secreting cell.

**SUMMARY**

The relationship between receptor molecules on antigen-binding lymphocytes (ABC) and antibody produced by antibody-secreting cells was studied in inbred strains of mice using the immune response to phosphorylcholine (PC) as a model system. Splenic and lymph node lymphocytes of nonimmune mice possess rare lymphocytes which bind [125I]-labeled PC-bovine serum albumin. The frequency of PC-ABC increases after immunization and is paralleled by a rise in the frequency of PC-specific antibody-producing cells. Both of these responses are thymus independent. The receptors on these ABC display specificity for PC and are exclusively of the IgM class. In one of the strains, BALB/c, the receptors possess the same idiotypic and fine degree of specificity for PC.
and two of its analogues, glycerophosphorylcholine and choline, that are characteristic of a PC-binding myeloma, HOPC 8. Furthermore, the idiotype and class of the receptor in these mice do not change during the course of the immune response. These data provide more direct evidence for the immunologic relevance of receptor-bearing lymphocytes.

We are indebted to Dr. Alexander Lawton for his gift of antiserum and purified myeloma proteins. The able technical assistance of Suzanne Murphy was greatly appreciated.

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