# DIFFERENTIAL EXPRESSION OF AN EQUIVALENT CLONOTYPE AMONG BALB/c AND C57BL/6 MICE*

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Experimental systems in which antigens elicit different responses among genetically distinct strains of mice have provided excellent tools for the study of mechanisms which govern specific immune responses. The characterization of some humoral responses has been facilitated through the analysis of their clonal composition by anti-idiotypic sera, isoelectric focusing, and the delineation of fine specificities for closely related antigens. With respect to these criteria, interstrain differences have now been established for murine responses to several antigens; including dextran (1, 2), *p*-azophenylarsonate (3, 4), streptococcal group A carbohydrate (5, 6), (4-hydroxy-3-nitrophenyl) acetyl,(4-hydroxy-5-bromo-3-nitrophenyl) acetyl (7, 8), staphylococcal ribonuclease (9), and phosphorylcholine (PC)[1] (10, 11). In many of these studies, the expression of a particular defined specificity in serum antibodies has correlated closely with the presence of a given heavy-chain allotypic ($C_H$) marker (12). These findings have been interpreted by some investigators as a reflection of linkage of $C_H$ and variable region ($V_H$) structural genes. As stated by these investigators, however, a fundamental and still unresolved interpretive difficulty exists in such examinations: Is the apparent linkage of $C_H$ and $V_H$ determinants indicated by serum antibody studies actually a result of $V_H$ structural gene differences, or may some of these allotype-linked expressions reflect instead, regulatory phenomena which act during or before antigenic stimulation?

This interpretive difficulty stems in part from the ambiguity inherent in the examination of heterogeneous serum antibody populations. In most serum antibody analyses, the designation of a strain as positive or negative for a given clonotype rests upon the degree of reactivity observed with an anti-idiotypic serum. Close scrutiny of such systems reveals that interstrain distinctions are rarely absolute. Rather, various degrees of cross-reactivity are observed. In a heterogeneous antibody mixture, idiotypic cross-reactivity may indicate either a diverse population of weakly cross-reactive antibodies or comparatively small quantities of the homologous idiotype. The resolution of this problem can only be accomplished through examination of homogeneous antibody populations. These may be obtained through either the fractionation of serum antibody components (13) or through the generation of monoclonal antibodies in vitro at limiting dilutions of precursor B cells (14). The latter method allows an enumeration of B cells of the relevant clonotype and thus enables the quantitative comparison of precursor

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[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; $C_H$, constant region of immunoglobulin heavy chain; DNP, 2,4-dinitrophenyl; Fab, fragment antigen binding; Hy, *Limulus polyphemus* hemocyanin; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PC, phosphorylcholine; TGG, tyrosylglycyl-glycine; RI, recombinant inbred; T15, TEPC 15 myeloma protein; $V_H$, variable region of immunoglobulin heavy chain.

population magnitude to serum expression. This type of comparison should allow a more definitive assessment of the clonotypes available to contribute to the net reactivity observed in serum antibody analyses, and it should provide insight with respect to the regulatory mechanisms which might act between the precursor cell and serum levels of clonotype expression.

Several aspects of the murine response to PC suggest an interplay of factors in the control of idiotype expression. The serum response of BALB/c and other strains of mice which bear the $a^1$ allotype is dominated by a clonotype identical to the TEPC 15 (T15) plasmacytoma protein (10). Strains which bear other allotypes, however, vary with respect to the expression of T15. Sera from A/J mice ($a^4$) display no anti-PC antibodies which are identical to T15 by serological criteria. C57BL/6 and other mice of the $a^2$ allotype, however, display a low level of cross-reactivity with T15 in both normal and immune sera (10).

Recently, the anti-PC response of several murine strains has been examined at the individual precursor cell level (15–18). The findings indicate that the majority of PC-responsive B cells in BALB/c mice are of the T15 clonotype. Further, strain AKR mice contain a minority of B cells which produce anti-PC antibodies indistinguishable from T15. C3H and A/J mice, however, display no clones whose products are identical to T15. Thus, the expression of the T15 clonotype at the precursor cell level is variable. Precursor cells of this clonotype can occur in allotypically distinct strains, although the degree of expression of this clonotype at the serum level appears to be closely associated with the Ig heavy-chain allotype markers. These findings suggest that the expression of a given clonotype may be regulated at several levels.

In the present study, we have examined several parameters which may reflect various levels of the regulation of clonotype expression. Both the serum and monoclonal anti-PC response of BALB/c mice, which express high levels of serum T15; and those of C57BL/6 mice, which express low levels of serum T15; as well as congenic and recombinant inbred (RI) strains from these parental types, have been examined. The results confirm the finding (10) that the T15 idiotype is readily detectable in the normal serum of only those mice which bear the $a^1$ allotype. When the PC-specific response of the B cells was analyzed in the splenic focus system, neither the number of PC-specific B cells nor the frequency of B cells which express the T15 clonotype within that population appeared to be related to allotypic markers. The strains tested could be divided into two groups with respect to the PC-specific precursor B-cell population. All strains bearing the BALB/c major histocompatibility complex (MHC) haplotype ($H\text{-}2^d$) displayed frequencies of anti-PC precursor B cells in the range of 15–20/$10^6$ splenic B cells. In contrast, all strains bearing the C57BL/6 MHC haplotype ($H\text{-}2^b$) exhibited anti-PC precursor frequencies in the range of 2–4/$10^6$ splenic B cells. The T15 clonotype was present at the precursor cell level in all strains, whether $a^1$ or $a^2$, and interstrain T15 frequency variations correlated with neither allotype nor MHC haplotype. Further, monoclonal antibodies from $a^2$ individuals could be shown to be indistinguishable from T15 in the idiotype assay employed.

These findings indicate that although both BALB/c and C57BL/6 mice express an equivalent clonotype at the precursor cell level and the frequency of these precursors is independent of allotype, the control of serum clonotype expression is allotype-associated.

## Materials and Methods

*Mice.* All mice used in these studies were 6–12 wk of age. BALB/c and A/He mice were obtained from the Institute for Cancer Research, Philadelphia, Pa. Bailey CXB RI mice (19, 20) and (BALB/c × C57BL/6)$F_1$ mice were bred in our animal colony from stocks originally obtained from The Jackson Laboratory, Bar Harbor, Maine. B10.D2 mice were purchased directly from The Jackson Laboratory, and CB20 mice (10) were obtained from Dr. Michael Potter, National Cancer Institute, National Institutes of Health, Bethesda, Md.

*Antigens.* The preparation and characterization of PC-tyrosylglycyl-glycine (TGG) and 2,4-dinitrophenyl (DNP) conjugates of *Limulus polyphemus* hemocyanin (Hy) and bovine serum albumin (BSA) have been described in detail previously (16, 21, 22).

*Diplococcus pneumoniae*, strain R36A, obtained from Dr. R. Austrian, University of Pennsylvania, Philadelphia, Pa., was heat-killed at 90°C for 60 min.

*Myeloma Proteins.* The BALB/c plasmacytomas T15, MOPC 167 (anti-PC), and MOPC 460 (anti-DNP) were obtained from Dr. Michael Potter at the National Cancer Institute. PC-binding myeloma proteins (23) were purified from ascitic fluid obtained from tumor-bearing mice as previously described (16) using a PC-Sepharose column prepared according to the method of Chesebro and Metzger (24).

*Immunizations.* (BALB/c × C57BL/6)$F_1$ mice were immunized intraperitoneally with 0.1 mg of Hy in complete Freund's adjuvant 6–10 wk before use as recipients in cell transfer experiments.

All mice used in serum antibody studies were exactly 6 wk of age. Mice were immunized to *D. pneumoniae* by a single intraperitoneal injection of $5 \times 10^8$ heat-killed organisms, and they were individually bled from the supraorbital sinus both immediately before, and 1 wk after immunization. Sera were stored frozen until assayed for anti-PC antibodies and T15 idiotype.

Anti-idiotype serum to T15 was prepared in A/He mice according to the protocol of Lieberman and Humphrey (25), and has been described in detail elsewhere (17). The serum obtained was passed over an MOPC 460 protein-Sepharose column to remove anti-allotype activity, and it was subsequently employed in the radioimmunoassay for the T15 idiotype.

*Splenic Focus Technique.* Spleen-cell preparations from donor mice were prepared by teasing the cells from the spleen in Dulbecco's modified Eagle's medium. The cells were washed once and resuspended in cold medium. 2–5 × $10^7$ viable cells were injected intravenously into Hy-immunized recipients that had been irradiated with 1,300 rad from a cesium source 4–6 h earlier. Fragment cultures were prepared from recipient spleens 16–18 h after cell transfer as described previously (26). The fragments were stimulated individually with PC-TGG-Hy ($1 \times 10^{-6}$ M hapten) or DNP-Hy, and culture fluids were changed every 3 days. Fluids collected 9–18 days after stimulation were assayed for anti-hapten antibodies and heavy-chain isotypes. Subsequently, the supernates from fragments which produced PC-specific antibodies were assayed for the T15 idiotype.

*Radioimmunoassay for Anti-Hapten Antibodies.* The assay of culture fluids for specific anti-PC or anti-DNP antibodies by a radioimmunoassay in which antigen (PC-BSA or DNP-BSA) is immobilized by adsorption to wells of polyvinyl plates has been described in detail elsewhere (14). Specific antibody was detected by purified, $^{125}$I-labeled goat anti-mouse-$\mu$, -$\gamma$, -$\alpha$, or rabbit anti-mouse fragment antigen binding (Fab).

*Radioimmunoassay for T15 Idiotype.* Idiotype assays were performed by a modification of previously reported procedures (15, 16). Briefly, the dilution of anti-idiotypic antibodies where control binding and inhibition were optimal was determined. Then, 0.1 ml of the appropriate dilution was added to wells of polyvinyl plates and incubated for 6 h at room temperature. The anti-idiotype serum was withdrawn, the plates were then washed once with phosphate-buffered saline (PBS), 0.2 ml of PBS containing 1% BSA (PBS-BSA) was added, and the plates were incubated for 30 min at room temperature. The PBS-BSA was removed and the plates were washed once with PBS. Subsequently, 20 $\mu$l of various concentrations of unlabeled standard inhibitor proteins, culture fluids, or serum dilutions were added, followed by 0.1 ml of PBS-BSA containing $\cong$2 ng of $^{125}$I-labeled T15 protein with a specific activity of 15–20 $\mu$Ci/$\mu$g. After an 18 h incubation at 37°C, the plates were washed six times with PBS and counted in a gamma counter.

*Iodination of Myeloma Proteins and Antisera.* Purified goat anti-mouse-$\mu$, -$\gamma$, and -$\alpha$ sera; purified rabbit anti-mouse Fab sera; and purified T15 protein preparations were iodinated by the

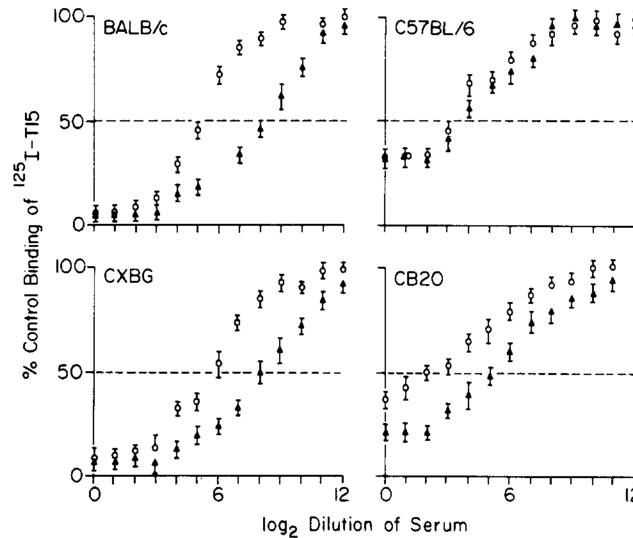


FIG. 1. Representative curves obtained from analysis of normal and immune sera for T15 reactivity. Individual mice were immunized with *D. pneumoniae* and bled immediately before, and 1 wk after immunization. Doubling dilutions of the serum were made in PBS-BSA and each dilution tested in triplicate in the T15 radioimmunoassay (see Materials and Methods), O, normal serum; ▲, immune serum. The coefficient of variation for such determinations was between 4 and 7%. Points are the means ± SD.

chloramine-T procedure with carrier-free $^{125}$I (Amersham/Searle Corp., Arlington Heights, Ill.) as described previously (21, 22).

## Results

*Quantitation of the T15 Idiotype in Normal and Immune Sera.* The results of an analysis of the T15 content of serum from normal and immune mice are shown in Fig. 1 and Table I. For each serum tested, the maximum possible inhibition of control $^{125}$I-T15 binding in the idiotype assay was determined. This parameter is essential for a critical appraisal of data obtained with heterogeneous antibody mixtures since maximum inhibition values of >90% probably reflect the presence of antibodies identical to T15, whereas values which fall below this level may indicate either extremely small quantities of antibodies identical to T15, or antibodies which only partially share determinants with T15. With this interpretive precaution in mind, the concentration of T15 or T15-like antibodies present in the serum was determined from the serum dilution at which 50% inhibition of control $^{125}$I-T15 binding was observed. This calculation, however, is based upon the assumption that the reactivity present in the serum is identical to T15 and must therefore be considered in conjunction with the maximum inhibition figures. Sera from mice of the $a^1$ allotype consistently inhibited 90% or more of $^{125}$I binding at low dilutions (Table I), and generally contained between 8 and 32 μg/ml of T15. Upon immunization, these strains exhibited five- to sixfold increases in the serum levels of T15; sometimes reaching concentrations of >100 μg/ml. Considerable variation in these levels was observed among mice of a given strain. Normal

TABLE I
*Analysis of Normal and Immune Sera for T15 Idiotype*

| Strain | Allotype | Immune status | Maximum inhibition observed | Serum* T15 concentration | |
|---|---|---|---|---|---|
| | | | | Mean | Range |
| | | | % | (μg/ml) | |
| BALB/c | $a^1$ | Before | 89 | 17 | 8–32 |
| | | After | 95 | 90 | 64–128 |
| CXBG | $a^1$ | Before | 90 | 13 | 8–32 |
| | | After | 90 | 90 | 64–128 |
| CXBJ | $a^1$ | Before | 92 | 24 | 8–32 |
| | | After | 90 | 83 | 32–128 |
| C57BL/6 | $a^2$ | Before | 65 | <2 | – |
| | | After | 55 | <2 | – |
| CXBD | $a^2$ | Before | 80 | <2 | <2–4 |
| | | After | 93 | 14 | 8–30 |
| CXBE | $a^2$ | Before | 60 | <2 | – |
| | | After | 60 | 4 | <2–8 |
| CXBH | $a^2$ | Before | 41 | <2 | – |
| | | After | 85 | 11 | 4–32 |
| CXBI | $a^2$ | Before | 47 | <2 | – |
| | | After | 60 | <2 | – |
| CXBK | $a^2$ | Before | 60 | <2 | – |
| | | After | 55 | <2 | – |
| CB20 | $a^2$ | Before | 65 | <2 | – |
| | | After | 85 | 4 | 2–8 |

* Serum concentration was calculated from the dilution at which 50% inhibition of control $^{125}$I-T15 binding was observed.

sera from mice of the $a^2$ allotype, however, never showed inhibition of $^{125}$I-T15 binding comparable to that observed with sera from mice of the $a^1$ allotype. Upon immunization, however, the $a^2$-bearing strains displayed various patterns. C57BL/6, CXBE, CXBI, and CXBK strains showed little or no increase in inhibitory capacity and maintained either extremely low or undetectable amounts of serum T15 based upon the dilution at which 50% inhibition of control binding was observed. CB20, CXBD, and CXBH mice, on the other hand, exhibited increases in inhibitory capacity to levels comparable to BALB/c individuals. In conjunction, these strains displayed detectable quantities of T15-like protein in their immune sera (Table I).

*Analysis of the Anti-PC Precursor Cell Frequency in RI Strains of BALB/c and C57BL/6 Origin.* Previous studies have shown that the frequency of primary anti-PC precursor B cells in the BALB/c mouse is 1 per 50,000–100,000 splenic B cells (15). In most other strains examined, the magnitude of the anti-PC specific precursor B-cell population is only slightly lower than that observed in BALB/c mice (17). Precursor cell frequency analysis of C57BL/6 mice, however, indicated that the anti-PC precursor frequency was 5- to 10-fold lower than in BALB/c individuals (Table II). It was thus of particular interest to examine the RI strains of these parental types to determine whether or not high and low frequency precursor characteristics segregated. Analysis of the Bailey CXB RI strains (Table II) indicated that these strains could be divided

TABLE II
*Summary of T15- and PC-Specific Precursors in BALB/c, C57BL/6, Recombinant Inbred, and Congenic Strains*

| Strain | H-2 | Allotype | PC-specific precursors per $10^6$ splenic B cells* | T15-positive precursors per $10^6$ splenic B cells |
|---|---|---|---|---|
| BALB/c | d | $a^1$ | 16 | 10 |
| CB20 | d | $a^2$ | 23 | 8 |
| B10.D2 | d | $a^2$ | 11 | 1 |
| CXBD | d | $a^2$ | 15 | 2 |
| CXBH | d | $a^2$ | 16 | 7 |
| CXBE | b | $a^2$ | 2 | 0.6 |
| CXBG | b | $a^1$ | 2 | 0.7 |
| CXBI | b | $a^2$ | 3 | 0.6 |
| CXBJ | b | $a^1$ | 3 | 2 |
| CXBK | b | $a^2$ | 4 | 0.6 |
| C57BL/6 | b | $a^2$ | 2 | 1 |
| (BALB/c × C57BL/6)$F_1$ | d/b | $a^1/a^2$ | 16 | 5 |

* Precursor frequencies given are after correction for homing efficiency of donor cells and cloning efficiency (14).

into two categories with respect to the magnitude of their anti-PC precursor frequency. CXBD and CXBH strains exhibited a high frequency of primary anti-PC precursors, similar to that of BALB/c. The other RI strains, however, displayed a low frequency comparable to that of C57BL/6 mice. The two high frequency strains were the only RI lines tested which possess the BALB/c MHC haplotype ($H\text{-}2^d$), whereas all other RI lines examined carry the C57BL/6 haplotype ($H\text{-}2^b$). These results suggested that elements which govern the frequency of anti-PC precursor cells might be linked to the MHC. To further investigate this possibility, the anti-PC response of the B10.D2 and CB20 congenic strains were analyzed. The data from such experiments support the association of high anti-PC precursor frequency with $H\text{-}2^d$ (Table II), as these mice display a relatively high frequency of primary anti-PC clones. (C57BL/6 × BALB/c)$F_1$ mice also show a high frequency of anti-PC precursor cells, suggesting that expression of high frequency is genetically dominant.

To eliminate trivial explanations for the observed differences in the anti-PC precursor frequency based upon donor-recipient interactions of an unknown nature, representative high and low frequency anti-PC strains were tested in the same experimental system for the anti-DNP response. Since the DNP response displays similar frequencies among all murine strains examined, including BALB/c and C57BL/6 (14, and unpublished observation), one would expect to obtain similar frequencies among the RI strains if the experimental system were examining only properties attributable to the donor B-cell population. The results from such experiments support this assumption, since very similar anti-DNP precursor cell frequencies were obtained in each of the strains tested.

*Idiotypic Analysis of Primary Anti-PC Monoclonal Antibodies from RI*

*Strains of BALB/c and C57BL/6 Origin.* The anti-PC response in BALB/c mice is dominated by the T15 clonotype (16), which comprises 40–80% of the BALB/c primary anti-PC repertoire. The presence of a predominant clonotype in the PC response is apparently unique to the BALB/c strain, since other strains examined do not appear to express one clonotype in such predominance (17). In the present experiments, the analysis of the T15 clonotype frequency has been extended to B10.D2 and CB20 congenic mice as well as the RI strains of BALB/c and C57BL/6 origin. Monoclonal antibodies which were obtained in the previously described experiments were tested using mouse anti-T15 serum in the competitive binding radioimmunoassay. The results of this analysis are shown in Table II, and reveal that the T15 clonotype is present in all tested strains, although at different frequencies. Furthermore, the relative frequency of the T15 clonotype in a given strain is associated with neither the MHC haplotype nor the Ig allotype of that strain.

*Characterization of T15-Positive Monoclonal Antibodies from $a^2$ Allotype Individuals.* Since the results demonstrate that the expression of T15 at the level of the individual precursor B cell is not allotype-linked, it was important to determine whether or not the monoclonal antibodies designated as T15-positive were in fact identical to the prototype T15 protein within the limits of the assay system. More specifically, we wished to find to what extent the results reflected partially shared determinants. Therefore, representative monoclonal antibodies, which had been designated T15-positive, were chosen and serial dilutions of these were examined in both the T15 and Fab radioimmunoassays. The results were compared to those obtained with purified T15 protein standards. The results (Fig. 2) indicate that even at high concentrations, the experimentally obtained monoclonal antibodies were indistinguishable from T15 by the antisera employed, and that the monoclonal antibodies could inhibit as much as 90% of the binding of the iodinated T15 to the anti-idiotypic serum.

## Discussion

We have examined the frequency and clonal composition of the primary anti-PC B-cell pool in C57BL/6, BALB/c, RI, and congenic strains derived from these parental types. The results of these studies and a detailed analysis of serum antibodies provide evidence which suggests that a complex set of regulatory phenomena influence precursor cell and serum antibody expression.

The frequency of PC-specific B cells in C57BL/6 mice is 5- to 10-fold lower than that observed in BALB/c mice. Moreover, anti-PC precursor cell frequency analysis of RI and congenic lines of BALB/c and C57BL/6 origin reveal that this characteristic follows the MHC in the strains examined. CXBH, CXBD, B10.D2, and CB20 strains (all $H\text{-}2^d$) exhibit a high PC precursor frequency, whereas all $H\text{-}2^b$ strains examined show a low precursor frequency. Whether or not this represents an actual linkage of regulatory elements to the H-2 complex, and whether or not the linkage can be attributed to subregions within the H-2 complex will require additional experiments with the appropriate congenic strains.

Analysis of the monoclonal anti-PC antibodies for the expression of the T15

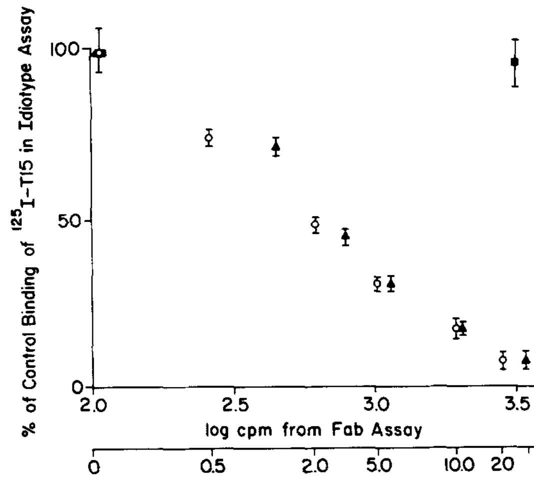


FIG. 2. Comparison of monoclonal antibody designated T15-positive and T15 myeloma protein in the T15 and Fab radioimmunoassays. Monoclonal antibody from mice of the $a^2$ allotype was serally diluted with PBS-BSA and tested in both the T15 and PC radioimmunoassays. Purified T15 at standard concentrations was tested in the same fashion. The points shown are means of at least four replicates. The coefficient of variation in the Fab assay was between 7 and 12%. Error bars show the SD of the cpm in the T15 assay. ○, T15 protein; ▲, monoclonal antibody 772 from CXBH mouse. MOPC 167 protein at a 20-μg concentration (■) was included to demonstrate the specificity of the assay system.

idiotype among the RI strains reveals that B cells which express the T15 clonotype are present in all strains examined, whether they are of the $a^1$ or $a^2$ allotype. Recently, Gearhart and Cebra (27) have also reported the presence of T15 precursors in CB20 and B10.D2 congenic strains, and they have shown that the IgA antibodies of this idiotype do not bear the $a^1$ allotype. Although the data presented here do not prove identity of the variable regions derived from the different strains, monoclonal antibodies from either $a^1$ or $a^2$ donors were not distinguishable from the T15 myeloma protein by the idiotype assay employed at either high or low concentrations.

Claflin and Davie (28) have described a combining site-associated idiotypic determinant on T15 which is detected by rabbit anti-T15 serum and which is found in immune sera of all murine strains tested. The anti-idiotypic serum used in this study and that used by Lieberman et al. (10), however, were raised in A/He mice, and since their binding is not appreciably inhibited by hapten, they probably detect variable region determinants outside the antibody-combining site. Thus, the presence of the T15 idiotype as analyzed in this report indicates identity with the T15 protein in the framework portions of the variable region, rather than a conserved combining-site determinant present in varying frameworks. It should be noted that representative monoclonal antibodies derived from either $a^1$ or $a^2$ mice and designated in this report as T15-positive by allogenic antiserum analysis were also indistinguishable by analysis with rabbit anti-T15 serum.

Thus, the expression of $a^1$ or $a^2$ allotype does not dictate the presence or

absence of the structural $V_H$ gene for T15, and appears to have little influence upon the frequency of T15 precursors within the B-cell repertoire. For example, BALB/c and CXBJ mice, both of the $a^1$ allotype, display high frequencies of the T15 clonotype, whereas CXBD mice, which are also $a^1$, show a comparatively low frequency of the T15 clonotype. Similarly, examples of both high and low T15 frequencies are observed among the strains bearing the $a^2$ allotype.

Previous reports (e.g. 10) have demonstrated that high levels of the T15 idiotype in normal or immune serum are closely associated with the presence of the $a^1$ allotype, and results in this report confirm this finding. One possible source of the apparent discrepancy between serum expression and precursor frequency might be that the mouse anti-T15 serum was directed against several determinants, all of which are present on the T15 molecule, and only some of which are present on the C57BL/6 anti-PC molecules which we have designated as T15-positive. This is probably not the case, however, since we have employed the criterion of a 1:1 weight relationship between the Fab and idiotype determinations of a particular monoclonal antibody in order for it be designated as T15-positive. Further, we have shown that similar inhibition curves may be obtained with either purified T15 protein or monoclonal antibodies designated as T15-positive, and these curves approach complete inhibition of $^{125}$I-T15 binding. It would be premature, however, to postulate complete identity between the monoclonal antibodies examined in this study and the T15 protein, since serological techniques may be insensitive to subtle differences which might be elucidated by amino acid sequence studies. It is more difficult to firmly establish close identity of antibodies in heterogeneous serum mixtures with the T15 protein. This factor is illustrated by the data presented herein with respect to serum levels of the T15 idiotype. Since complete inhibition of the $^{125}$I-T15 binding in the idiotype assay was observed only with sera from mice of the $a^1$ allotype, the lesser inhibition observed with serum from mice of $a^2$ allotype might be the result of cross-reactive antibody populations which share some T15 determinants. On the other hand, since it is clear from the analysis of monoclonal antibodies that all strains examined possess the T15 clonotype at the precursor level, it is equally possible that mice which bear the $a^2$ allotype express T15 in their sera, but at levels too low to produce complete inhibition. If this is the case, it would appear that C57BL/6 mice fail to use this clonotype in their response to R36A, whereas CB20 and CXBH mice do so to a limited extent. This result parallels the data of Lieberman et al. (10), who observed that the hemagglutination inhibition titers of T15 in the sera of CB20 and CXBH mice increased slightly upon immunization, whereas that of C57BL/6 mice did not.

Two critical points emerge from the findings reported here: (*a*) the allotype of an individual, although closely related to serum levels of an idiotype, is unrelated to the proportion of the precursor population which expresses that idiotype; and (*b*) the serum expression of a given idiotype may reflect regulatory processes more than the actual clonotype representation in the B-cell repertoire.

It is apparent from these findings that a distinction must be made between the expression of idiotypic determinants within precursor B-cell populations and elements which regulate the subsequent appearance of those idiotypes in

serum antibodies. This distinction places limitations upon the inferences which may be drawn from either the examination of in vivo serum antibody or the study of precursor B-cell populations in vitro. Foremost among these limitations is the extent to which such data may be used to assess the structural gene complement of an individual or a strain. Thus, the examination of serum antibody may be misleading if it is assumed to be representative of the complete $V_H$ gene repertoire. Analysis of precursor B-cell populations stimulated in vitro, on the other hand, overcomes many ambiguities inherent in the examination of serum antibodies and thus allows a more thorough assessment of the structural gene complement of an individual. However, such analyses cannot necessarily be used for the accurate prediction of the antibodies which will be elicited during an in vivo immune response. It is imperative, therefore, that results obtained from each system be interpreted within the context of these limitations.

One distinction between the regulation of precursor population magnitude and the allotype-associated regulation of serum antibody levels may be that the latter appears to be antigen-driven whereas the former is not. For example, previous reports from this laboratory have shown that the T15 precursor population in germ-free mice is similar to that observed in conventionally reared mice. In contrast, germ-free BALB/c mice do not express T15 in their sera, but they may be induced to do so upon exposure to a conventional environment (10). This distinction would suggest that the development of B cells which express various $V_H$ determinants is relatively independent of external influences, but the capacity of these cells to proliferate and secrete antibodies will be dictated by other regulatory mechanisms.

The observed dichotomy between serum expression and precursor cell frequency also places constraints upon the designation of a given clonotype as a germline antibody specificity. Although the T15 clonotype is considered to be encoded in the germline because of its high frequency in all BALB/c individuals, it is present at low frequency in some RI strains. Moreover, CB20 and CXBH mice possess populations of T15 precursor cells whose magnitude is not directly reflected in either normal or immune sera. Clonotypes present at very low frequency have been described in other systems (13), but have generally been interpreted as a reflection of somatically generated specificities, since one criterion for a germline specificity has been its presence in all individuals of a particular strain. Since the T15 clonotype is generally accepted as a germline specificity, the present observations suggest that at least some minor (low frequency) clonotypes are encoded by germline information, and that the distinction between major and minor clonotypes at the serum antibody level might reflect differences in genetic background, rather than $V_H$ gene differences. It will now be of importance to examine these possibilities in detail and to determine whether or not other idiotypic systems display the same dichotomy between specificities which are expressed within precursor populations and those which are observed in serum antibodies.

## Summary

The primary anti-phosphorylcholine (PC) response in BALB/c, C57BL/6, and congenic and recombinant inbred strains of these parental types has been

examined in the splenic focus system. The frequencies of PC-specific precursors were shown to vary among these strains from 2 to 20 precursors per $10^6$ splenic B cells. The distribution of these frequencies suggests that elements closely linked to or within the major histocompatibility complex may play a role in the determination of this parameter, although additional experiments are necessary to adequately assess this possibility. Moreover, all strains tested, regardless of immunoglobulin allotype, expressed monoclonal antibodies indistinguishable from the TEPC 15 myeloma protein (T15) clonotype. Further, the frequency of this clonotype in a given strain did not appear related to allotype, since both high and low T15 frequencies were found among strains of either the BALB/c ($a^1$) or C57BL/6 ($a^2$) allotype. The examination of normal serum for the T15 idiotype, however, revealed that only mice of the BALB/c allotype ($a^1$) expressed the T15 idiotype in detectable quantities. After immunization with *Diplococcus pneumoniae*, sera from mice of the $a^1$ allotype consistently contained large quantities of the T15 idiotype, whereas sera from mice of the $a^2$ allotype exhibited various degrees of cross-reactivity with anti-T15 antibody.

These results suggest that: (*a*) the allotype of an individual, although closely related to serum levels of an idiotype, is unrelated to the proportion of the precursor population which expresses that idiotype and; (*b*) the serum expression of a given idiotype may reflect regulatory processes, which act either during or before antigenic stimulation, rather than the actual clonotype representation in the repertoire. These findings indicate that distinctions must be made between the expression of idiotypic determinants within precursor B-cell populations and elements which regulate the subsequent appearance of those idiotypes in serum antibodies.

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