ROLE OF A NONIMMUNOGLOBULIN CELL SURFACE DETERMINANT IN THE ACTIVATION OF B LYMPHOCYTES BY THYMUS-INDEPENDENT ANTIGENS*

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Thymus-independent (TI)1 antigens provide powerful tools to elucidate the requirements for specific antigen-induced triggering of B lymphocytes. Recently it has been proposed that TI antigens can be divided into two groups (1, 2). TI antigens that stimulate substantial responses by B cells from both immunologically defective CBA/N mice and normal neonatal mice, such as trinitrophenylated Brucella abortus (TNP-BA), are designated as TI-1 antigens (1-3). In contrast, TI-2 antigens evoke antibody responses only by B cells from normal adult mice but not by B cells from adult CBA/N or normal neonatal mice. Examples of TI-2 antigens are trinitrophenyl aminoethylcarbamyl-methyl Ficoll (TNP-FicolI), polyinosinic-polycytidilic acid, dextrans and pneumococcal polysaccharides (4-7). This classification of TI antigens correlates well with the observations that B lymphocytes from neonatal mice and from the immunodeficient CBA/N mice show certain common surface phenotypic and functional characteristics. These properties distinguish such B cells from one subset of B cells found in the spleens of normal adult mice (8-11). The presence of at least two phenotypically distinct subpopulations of murine B cells raises the possibility that the differential stimulatory capacity of TI-1 and TI-2 antigens is due to differences in the triggering requirements of B-cell subpopulations (1, 12). According to this view, TI-2 antigens activate only that subset of B lymphocytes which is unique to adult mice while TI-1 antigens stimulate either only the early developing (CBA/N like) B cells or both early and late-appearing subsets of B lymphocytes.

We have recently prepared an alloantisemur (anti-Lyb 5.1) which is cytotoxic for 60% of B lymphocytes from adult mice and therefore divides B cells into Lyb5+ and Lyb5− subpopulations (13). In the course of analyzing the functional properties of

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1 Abbreviations used in this paper: PAGE, polyacrylamide gel electrophoresis; PC-BA, Brucella abortus conjugated with phosphorylcholine; PFC, plaque-forming cell; SDS, sodium dodecyl sulfate; TI, thymus independent; TNP-BA, trinitrophenylated Brucella abortus; TNP-FicolI, trinitrophenylated aminoethylcarbamylmethyl Ficoll.

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Lyb5+ and Lyb5− subpopulations of normal adult B lymphocytes, we have observed that anti-Lyb 5.1 serum, if present in the culture continuously, inhibits the in vitro anti-TNP antibody response to the TI-2 antigens such as TNP-Ficoll and TNP-dextran. This serum does not inhibit the in vitro anti-TNP antibody response to optimal concentrations of the TI-1 antigens, TNP-BA and TNP-lipopolysaccharide. The ability of anti-Lyb 5.1 serum to inhibit antibody responses to TI-2 antigens suggests that it recognizes a cell surface determinant(s) which plays an important role in the activation of B cells by TI-2 antigens. However, the blocking activity of anti-Lyb 5.1 serum appears to be distinct from the cytotoxic activity of this antiserum, since the cells of AL/N mice are sensitive to the cytotoxic activity but resistant to the blocking activity. Furthermore, data to be presented in a subsequent paper will show that the gene(s) specifying Lyb 5.1 segregate independently from the gene(s) responsible for the alloantigen involved in the in vitro blocking of TI-2 antibody responses.  

Materials and Methods

Animals. DBA/2N, C57BL/6N, AL/N, C3H/HeN, BALB/cAnN, B10.D2/nSnN, (CBA/N × DBA/2)F1, and (DBA/2N × CBA/N)F1 mice were obtained from the Small Animal Section, Division of Research Services, National Institutes of Health. The immune defect of CBA/N mice is X-linked; F1 male mice of a cross between CBA/N females and DBA/2 males [(CBA/N × DBA/2)F1] are hemizygous for the defective gene and are functionally defective. The females from this cross and the reciprocal hybrid (DBA/2 × CBA/N) are heterozygous for the defective gene and are phenotypically normal. The male offspring of this latter cross, i.e. (DBA/2 × CBA/N) derive their X-chromosome from the DBA/2 mothers and hence are normal. DBA/J, SWR/J, CE/J, AKR/J, C57BL/6J, RF/J, B10.D2/nSn, A/J strains of mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Cell Culture. Spleen cells were cultured by the method of Mishell and Dutton (14) with the modifications as described previously (15). Single cell suspensions of mouse spleen cells were cultured in 96 well flat bottom microtitler plates (Linbro FB-96 TC, Linbro Scientific Co., Hamden, Conn.; or 3596, Costar, Cambridge, Mass.). 1 million cells were cultured in 0.2 ml medium under stationary conditions in a humidified 5% CO2, 95% air atmosphere at 37°C. The test or control antisera were present at 1% final concentration throughout the culture period.

Antigens. TNP-Ficoll and TNP-dextran were prepared as described by Inman (16) from aminoethylcarbamylmethyl derivatives of Ficoll and dextran T-500, respectively. Ficoll (average mol wt - 400,000 daltons) and dextran T-500 were obtained from Pharmacia Fine Chemicals, Piscataway, N. J. TNP-Ficoll had 83 aminoethylcarbamylmethyl groups and 56 TNP groups per mole of Ficoll. TNP-BA was prepared by the method of Mond et al. (3). The stock suspension used as starting material had 10⁶ microorganisms per milliliter. The concentrations of this antigen expressed in this paper are dilutions of the stock. TNP-lipopolysaccharide from Escherichia coli 0111: B4 (TNP-LPS) was prepared according to the method of Jacobs and Morrison (17). Phosphorylcholine was coupled to B. abortus by a method analogous to that described by Chesebro and Metzger (18).

Plaque-Forming Cell (PFC) Assay. The cultures were assayed on day 4 unless mentioned otherwise. The number of direct PFC was assayed by using a glass slide modification of the hemolytic plaque assay of Jerne and Nordin (19). Sheep erythrocytes, coupled with TNP by the method of Rittenberg and Pratt (20), were used as indicator cells when cultures were stimulated with antigens having TNP as hapten. In the experiments in which PC-BA was the stimulating antigen, sheep erythrocytes coated with pneumococcal extract (21) by the method of Gold and Fudenberg (22) were used as target cells. The results are expressed as the geometric mean PFC per 10⁶ cultured cells ×/+ relative SE of triplicate groups.

Subbarao, B., A. Ahmed, W. E. Paul, I. Scher, R. Lieberman, and D. E. Mosier. 1978. Lyb7, a B cell alloantigen controlled by genes linked to the IgG4 locus. Manuscript submitted for publication.
**Cell Surface Iodination, Solubilization, and Immunoprecipitation.** The procedure of Kessler (23) was employed. It consisted of labeling 50 × 10⁶ spleen cells, depleted of erythrocytes by hypotonic lysis, with 1 μCi of Na¹²¹I in the presence of 1–5 I U of lactoperoxidase (grade B, sp act 42 I U/mg; Calbiochem, San Diego, Calif.) and reagent grade hydrogen peroxide. The washed cells were solubilized with 0.5% NP-40 and centrifuged at 110,000 g for 30 min. The supernate was preabsorbed for 30 min with 50 μl of 10% suspension (vol/vol) of *Staphylococcus aureus* Cowan I strain (ATCC 12598) for a lysate obtained from 25 × 10⁶ cells. Immunoprecipitation was done by the method of Cullen and Schwartz (24).

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).** The procedure of Laemli (25) as modified by Cullen and Schwartz (24) was employed. Samples were reduced with 2-mercaptoethanol in the presence of SDS and applied to 10% polyacrylamide gels containing 0.1% SDS. Electrophoresis was carried out at a current of 5 mA/gel.

**Antisera.** Anti-Lyb5.1 serum was prepared according to the procedure of Ahmed et al. (13). Briefly, hyperimmune C57BL/6 anti DBA/2 spleen cell antiserum was extensively absorbed with DBA/2 thymocytes until it had no anti-H-2d activity. The antiserum was then absorbed with spleen cells from B-cell defective (CBA/N × DBA/2)F₁ male mice until it had no cytotoxicity against the same cells. At this stage the antiserum still had an ability to kill 20–25% of the spleen cells from (CBA/N × DBA/2)F₁ female mice or from DBA/2 mice and usually had cytotoxic titers of 1:16 to 1:32. All strains that are negative in this cytotoxicity test are referred to as Lyb5.1 negative strains, because it is not known if there are more than two allelic forms of Lyb 5.

Control antiserum was prepared by absorbing anti-Lyb5.1 serum with spleen cells from DBA/2 mice until it had no cytotoxicity against the same cells. Absorptions were carried out by addition of the antiserum to packed spleen cells and incubation at 4°C on rotary shaker for 1 h. All antisera were deaggregated just before use by ultracentrifugation at 130,000 g for 30 min in an airfuge (Beckman Instruments Inc., Cedar Grove, N. J.).

Rabbit anti-mouse κ used in immunoprecipitation studies was a generous gift from Dr. Rose Mage (Laboratory of Immunology; NIAID, NIH). C3H/HeJ anti-CBA/J and CBA/J anti-C3H/HeJ sera were kindly provided by Dr. D. H. Sachs (Immunology Branch, NCI, NIH). These sera had cytotoxic titers of at least 1:64.

**Cytotoxic Assay.** 2.5 × 10⁵ spleen cells depleted of erythrocytes were incubated with 50 μl of antiserum diluted in 50 μl of RPMI-1640 + 5% fetal calf serum in a microtiter plate at 4°C. After 1 h the plate was centrifuged at 800 g for 10 min and the supernate was discarded. Cells were incubated with 100 μl of a 1:10 dilution of rabbit complement at 37°C for 30 min. The rabbit serum used as complement source had been screened for low background killing. The viability of the cells was tested by their ability to exclude trypan blue.

**Results**

**Effect of Anti-Lyb 5.1 Serum on In Vitro PFC Responses.** Spleen cells from immunologically normal (DBA/2 × CBA/N)F₁ male mice were cultured with TNP-BA (10⁻² dilution of stock) or TNP-Ficoll (10⁻³ μg/ml) in the continuous presence of a 1% concentration of anti-Lyb 5.1 or control serum. No external source of complement was added. 4 d later the number of direct anti-TNP PFC were determined. A representative experiment of this type is shown in Table I. The PFC responses to TNP-Ficoll were inhibited by more than 80% while there was little or no effect on the PFC response to this concentration of TNP-BA.

**Specificity of the Inhibition of Responses to TNP-Ficoll by Anti-Lyb 5.1 Serum.** If the inhibition of PFC responses to TNP-Ficoll is a specific effect due to alloantibodies present in this antiserum, then inhibition should be observed only with those strains that have been typed as Lyb 5.1 positive by the cytotoxicity test but should not be observed with those strains that do not carry the Lyb 5.1 allele. In 20 individual experiments, responses of DBA/2 (Lyb 5.1⁺) cells to TNP-Ficoll were inhibited by 62%, whereas responses of C57BL/6 (Lyb 5.1⁻) cells to TNP-Ficoll were not signifi-
**TABLE I**

Inhibition of PFC Responses to TNP-Ficoll by Anti-Lyb 5.1 Serum

|                | Control serum | Anti-Lyb 5.1 | Inhibition |
|----------------|---------------|--------------|------------|
| **PFC/10⁶ Cells** |               |              | %          |
| TNP-BA§        | 410‡          | 354          | 14         |
| (1.14)         | (1.48)        |              |            |
| TNP-Ficoll     | 192           | 30           | 84         |
| (1.05)         | (1.02)        |              |            |

* (DBA/2 × CBA/N)F₁ male spleen cells were cultured with the antigens in the presence of a 1% concentration of control serum (Materials and Methods) or anti-Lyb 5.1.

§ Background PFCs which were <20 are subtracted. The values are expressed as geometric means (×/± relative SE).

§ TNP-BA was used at a 1:200 dilution of the stock concentration. TNP-Ficoll was used at 10⁻⁶ μg/ml.

**TABLE II**

Strain Specificity of Inhibition of PFC Responses to TNP-Ficoll by Anti-Lyb 5.1 Serum

| Strain          | Percent inhibition of PFC response to* |
|-----------------|----------------------------------------|
|                 | TNP-Ficoll | TNP-BA |
| DBA/2           | 62 ± 4     | 2 ± 8  |
| (20)            | (19)       |        |
| C57BL/6         | 14 ± 6     | -14 ± 10 |
| (8)             | (8)        |        |
| Lyb 5.1-Positive strains‡ | 63 ± 3 | -1 ± 7 |
| (40)            | (41)       |        |
| Lyb 5.1-Negative strains§ | 8 ± 4 | -16 ± 8 |
| (14)            | (11)       |        |
| (CBA/N × DBA/2)F₁ Female|| 74 ± 5 | 3 ± 16 |
| (7)             | (6)        |        |

* Results are expressed as Mean ± SE. The numbers within the parentheses indicate the number of observations. Negative numbers signify enhancement.

‡ DBA/2, DBA/1, CE/J, (CBA/N × DBA/2)F₁ female, (DBA/2 × CBA/N)F₁ male, C3H/HeJ.

§ BALB/c, C57BL/6, CBA/J, SJL, A/J, AKR/J, B10.D2, RF/J.

‖ Because CBA/J and CBA/CaJ mice are negative in both the cytotoxicity and blocking tests, the (CBA/N × DBA/2)F₁ female mice are very likely to be heterozygous for the alloantigens recognized by anti-Lyb 5.1 serum.

Significantly inhibited (Table II). Responses to TNP-BA were not blocked in either strain. Summary data from experiments with several other Lyb 5.1-positive and negative strains are included in Table II. These data demonstrate that the responses of Lyb 5.1 negative strains to TNP-Ficoll were not affected by this antiserum. Furthermore, the responses to TNP-Ficoll by Lyb 5.1 positive-strains listed in Table II were inhibited to approximately the same degree as the response of DBA/2 mice. This includes mice which are heterozygous for Lyb 5.1.

As an additional control for the significance of the blocking effect, we have tested another B-cell alloantiserum, anti-LyM 1 (CBA/J anti-C3H/HeJ) serum (26) in our
system and found that this serum had no inhibitory effect on the responses to either TNP-Ficoll or TNP-BA (Table III). This suggests that the inhibition caused by anti-Lyb 5 is not a simple consequence of the binding of antibody to any membrane antigen of B lymphocytes.

A detailed strain distribution of the cytotoxic and blocking activities of anti-Lyb 5.1 serum is presented in Table IV. The failure of anti-Lyb 5.1 serum to block responses of AL/N lymphocytes despite its capacity to lyse these cells provides initial evidence that the blocking antibodies and the cytotoxic antibodies may be directed against distinct alloantigens. This will be discussed in more detail later and in a subsequent paper. For clarity, we will refer to the ability of anti-Lyb 5.1 serum to selectively inhibit responses to TNP-Ficoll as its blocking activity.

Susceptibility of PFC Responses to Various TI-1 and TI-2 Antigens to Inhibition by Anti-Lyb 5.1 Serum. We observed that the extent of inhibition by anti-Lyb 5 serum was approximately the same, over a wide range of concentrations \((10^{-2} - 10^{-5} \text{ mg/ml})\) of TNP-Ficoll (Fig. 1). Similarly, this antiserum inhibited the PFC responses to another TI-2 antigen, TNP-dextran, at all antigen concentrations tested (Fig. 2). In contrast, the effect of anti-Lyb 5.1 serum on PFC responses to TNP-BA was dependent on the concentration of the antigen used (Table V). At the concentration of TNP-BA...
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![Graph](image)

**Fig. 1.** Effect of anti-Lyb 5.1 serum on PFC responses to different concentrations of TNP-Ficoll. Control serum is an anti-Lyb 5.1 serum that has been absorbed with spleen cells from DBA/2 mice until it has neither cytotoxic nor blocking activity. The antisera were used at 1% concentration. DBA/1 spleen cells were cultured in Mishell-Dutton cultures in the presence of indicated antigen concentrations and control serum (●) or anti-Lyb 5.1 (○) serum.

![Graph](image)

**Fig. 2.** Effect of anti-Lyb 5.1 serum on in vitro primary responses to TNP-dextran. DBA/2 spleen cells were cultured in presence of control serum (●) or anti-Lyb 5.1 (○) serum. Other conditions are the same as described in legend to Fig. 1.

employed in the experiments thus far described (10^{-2} dilution of the stock), anti-Lyb 5.1 caused no significant inhibition of the anti-TNP response. However, at lower concentrations of TNP-BA (10^{-4} or lower) the responses are substantially diminished in presence of anti-Lyb 5.1 serum. It is interesting to note that CBA/N mice respond poorly to such low doses of TNP-BA (3).

Anti-Lyb 5.1 serum does not affect the PFC responses to TNP-LPS, another TI-1 antigen, at all the concentrations of the antigen tested (1-100 μg/ml). However, responses to a wide dose range of PC-BA are suppressed by anti-Lyb 5.1 antiserum (Table VI). PC-BA belongs to the TI-2 class of antigens since the mice with CBA/N
TABLE V
Effect of Anti-Lyb 5.1 Serum on PFC Responses to Different Concentrations of TNP-B. abortus

| Concentration* | Anti-TNP PFC/10⁶ cells in presence of: | Inhibition | Mean percent inhibition ± SE‡ |
|----------------|--------------------------------------|------------|------------------------------|
|                | Control serum                       | Anti-Lyb 5.1 serum |                      |
| 1:10²          | 508                                  | 526        | -4                          | 15 ± 17          |
|                | (1.01)                               | (1.07)     |                             |                  |
| 1:10⁴          | 308                                  | 86         | 72                          | 67 ± 10          |
|                | (1.11)                               | (1.18)     |                             |                  |
| 1:10⁶          | 166                                  | 24         | 84                          | 64 ± 24          |
|                | (1.19)                               | (1.84)     |                             |                  |

* Concentrations are expressed as dilutions of the stock, which has 10⁸ micro-organisms per ml.
‡ The values are average results from three experiments.

TABLE VI
Effect of Anti-Lyb 5.1 Serum on Antibody Responses to TI-1 and TI-2 Antigens

| Antigen*       | Ability of CBA/N mice to respond | Inhibition by anti-Lyb 5.1‡ | Class of the antigen |
|----------------|----------------------------------|----------------------------|----------------------|
| TNP-BA         | +                                | -1 ± 7 (41)                | TI-1                 |
| TNP-LPS        | +                                | 11 ± 10 (2)                | TI-1                 |
| TNP-Ficoll     | -                                | 63 ± 3 (40)                | TI-2                 |
| TNP-Dextran    | -                                | 68 ± 9 (3)                 | TI-2                 |
| PC-BA          | -                                | 71 ± 5 (7)                 | TI-2                 |

* TNP-BA and PC-BA were used at 1:200 and 1:1,000 dilution of the stock, respectively. The stock solutions of TNP-BA and PC-BA, respectively, contained 10⁸ and 5 × 10⁶ micro-organisms per ml.
Concentrations of TNP-LPS, TNP-Ficoll, and TNP-dextran in that order are 10 µg/ml, 10⁻³ µg/ml, and 10⁻⁴ µg/ml. PFC responses were measured 4 d after initiation of the cultures and they varied from 80 to 400 PFC per 1 million cultured cells.
‡ The values represent mean ± SE with the number of experiments given in the parentheses.

immune defect do not respond to phosphorylcholine on any carrier including BA (27). Thus, the responses to the three TI-2 antigens tested are susceptible to inhibition by anti-Lyb 5.1 serum. Responses to optimal concentrations of two TI-1 antigens are not inhibited, although anti-Lyb 5.1 serum does diminish the response to a low concentration of TNP-BA.

Timing of Addition of Anti-Lyb 5.1 Serum to the Cell Cultures. As a preliminary step in the analysis of the mechanism by which anti-Lyb 5.1 serum inhibited B cell responses, we wished to know when the antiserum had to be added to obtain inhibition. To test this we added anti-Lyb 5.1 or control serum to the culture at different times after the addition of TNP-Ficoll. Results of one such experiment are presented in Fig. 3. Anti-Lyb 5.1 caused significant inhibition when added as late as 14 h after initiation of culture; when added at later times, little or no inhibition was observed. The loss of susceptibility to inhibition may be due either to a loss of the Lyb 5.1 antigen from the
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FIG. 3. DBA/1 spleen cells were cultured in presence of $10^{-4} \mu g/ml$ concentration of TNP-Ficoll. Control serum and anti-Lyb 5.1 serum were added to parallel cultures at the indicated times after initiation of the cultures. PFC responses were assayed 4 d later. In this experiment response in presence of control serum was 252 PFC/10^6 cells and it was taken as 100%.

FIG. 4. SDS-polyacrylamide gel electrophoresis of 125I labeled (DBA/2 × CBA/N-)F1 male spleen cell membrane proteins precipitated by rabbit anti-mouse light-chain serum (A) and by anti-Lyb 5.1 serum (B). Precipitates were reduced by boiling in 5% 2-mercaptoethanol and 2% SDS and were subjected to electrophoresis in 10% polyacrylamide gels with 0.1% SDS. 2-mm slices were cut and the radioactivity was measured.

Nature of Cell Surface Molecules Recognized by Anti-Lyb 5.1 Serum. It has been shown previously that an alloanti-δ serum also can inhibit in vitro antibody responses to TNP-Ficoll (28). Spleen cells from CBA/N or (CBA/N × DBA/2)F1 male mice have an sIgD to sIgM ratio which is approximately ½ that of spleen cells from normal mice (9). Furthermore, Lyb 5-negative cells from normal adult mice have a surface Ig phenotype similar to that of CBA/N cells, i.e. sIgM > sIgD (13). Hence it is a formal possibility that C57BL/6 anti-DBA/2 serum contains anti-δ antibody and that absorption with (CBA/N × DBA/2)F1 male spleen cells which may be low in sIgD density could have failed to completely remove anti-δ activity. To test this possibility, cells were surface labeled with 125I and the solubilized membrane proteins were subjected to immunoprecipitation with anti-Lyb 5.1 serum and S. aureus. The resultant precipitates were dissolved in 2% SDS and were analyzed on SDS-PAGE as described. Fig. 4B shows the result of such an experiment. For comparison, SDS-PAGE pattern of an immunoprecipitate obtained with rabbit anti-mouse-κ is presented in Fig. 4A.
It is clear that there are no molecules in the material reactive with anti-Lyb 5.1 that migrate in the position of \( \mu \) (1.8 cm), \( \delta \) (2.5 cm), or \( \gamma \) (3.9 cm) heavy chains. The pattern obtained with anti-Lyb 5.1 is complex and it is difficult to conclude which of the peaks represents the Lyb 5.1 molecules. This result is not surprising since our results suggest that at least two antibodies are present in anti-Lyb 5.1 serum, each of which is directed against a different cell surface antigen.\(^2\)

Discussion

In this paper we have demonstrated that anti-Lyb 5.1, an alloantiserum which recognizes a mature subset of B lymphocytes, selectively inhibits in vitro PFC responses to TI-2 antigens, but does not effect responses to optimal concentrations of TI-1 antigens. This selective inhibition was seen in four Lyb 5.1-positive strains (DBA/2, DBA/1, C3H/HeJ and CE/J). None of the Lyb 5.1-negative strains gave responses to TNP-Ficoll that were susceptible to inhibition by the presence of anti-Lyb 5.1 serum during the culture period. However, there is one strain, AL/N, in which responses to TNP-Ficoll are not affected by anti-Lyb 5.1 serum even though AL/N mice are positive for Lyb 5.1 expression by the cytotoxicity test. This finding suggested that the inhibitory and cytotoxic activities of anti-Lyb 5.1 serum might be due to two different specificities present in one antiserum. In a subsequent paper\(^2\) we show that these two activities (blocking and cytotoxicity) segregate independently in the mice from the backcross (C57BL/6 \( \times \) DBA/2) \( \times \) C57BL/6. Thus the cytotoxic and blocking activities of this alloantiserum are due to recognition of two different cell surface determinants which are encoded by unlinked genes.

For the sake of clarity in this discussion, we will refer to the specific antigen recognized by the blocking activity of this antiserum as Lyb 7.1 as opposed to Lyb 5.1 which was defined by the cytotoxic activity of the antiserum. In the following discussion, any reference to anti-Lyb 7.1 is only to this blocking activity present in the anti-Lyb 5.1 serum since all the experiments reported in this paper make use of anti-Lyb 5.1 serum. Anti-Lyb 7.1 activity is present in an antiserum that has been extensively absorbed with spleen cells from the (CBA/N \( \times \) DBA/2)\( F_1 \) male mice, strongly suggesting that Lyb 7.1 is also present only on a subset of B lymphocytes that are absent from mice with the CBA/N defect. Since there are no Lyb 5\(^+\) cells in CBA/N mice, it is likely that Lyb 7 is present exclusively on Lyb 5\(^+\) B cells. The designation Lyb 7.1 is a preliminary one until decisive studies on tissue distribution are completed.

In vitro response to TI-2 antigens are always significantly suppressed by anti-Lyb 7.1, but the inhibition was never total, the average inhibition being 62%. It is conceivable that this reflects a low titer of the antibody, especially since the inhibition falls off rapidly at greater dilution (data not shown). However, since similar results were obtained with many different batches of the antiserum, it is formally possible that there is further heterogeneity among the B lymphocytes that respond to TI-2 antigens. At present we are making efforts to produce these antibodies in higher titers by alternative approaches.

Although the anti-TNP antibody response to optimal concentrations of TNP-BA is not suppressed by anti-Lyb 7.1 antibodies, there is significant inhibition of responses to lower concentrations of this antigen. As mentioned above, mice with the CBA/N defect respond poorly to such low doses of TNP-BA while giving normal responses to optimal concentrations of this antigen. One interpretation of this result is that low
concentrations of TNP-BA stimulate only Lyb 7+ cells and the responses of such cells are inhibited by anti-Lyb 7.1 serum. It is likely that both Lyb 7+ and Lyb 7− cells are involved in responses to high doses of TNP-BA and the failure to observe any inhibition at these concentrations may be the result of a relative insensitivity of the present culture system to changes in precursor frequency. One prediction of this hypothesis is that anti-Lyb 7.1 activity will inhibit responses of Lyb 7+ cells to any antigen. An alternative possibility is that Lyb 7+ cells are involved in responses of adult mice to TNP-BA at both high and low doses but that only the responses to low concentrations are susceptible to inhibition by anti-Lyb 7.1. This issue can be resolved by an analysis of the effect of anti-Lyb 7.1 serum on both precursor frequency and burst size in responses to high and low concentrations of TI-1 antigens.

Our experiments do not address directly the question of the mechanism of the inhibition of responses to TI-2 antigens by anti-Lyb 7.1 antibodies. Since two alloantisera that have been tested, namely anti-δ (28) and anti-Lyb 7.1, both inhibit the responses to TNP-Ficoll, it is conceivable that a perturbation of the cell membrane due to interaction of any antiserum with a cell surface component may inhibit the response of B cells to this class of antigens. The inability of CBA/J anti-C3H/HeJ serum, which is cytotoxic for B cells (26), to cause any inhibition of the responses to TNP-Ficoll constitutes strong evidence against this possibility. Similarly as will be shown subsequently, anti-Lyb 5.1 serum fails to inhibit responses to Lyb 5.1+, Lyb 7.1− backcross mice.

Another explanation of the inhibition is that binding of anti-Lyb 7.1 to Lyb 7 molecules on the cell surface sterically inhibits any effective interaction of antigen with the sIg receptors or some mitogen receptors (29). Since the responses of spleen cells from an animal homozygous or heterozygous for Lyb 7 are equally sensitive to inhibition, such a mechanism for anti-Lyb 7.1 action seems unlikely. This argument presumes that in the heterozygous mice, half of the Lyb 7 molecules remain unoccupied by the antibodies directed against the Lyb 7.1 determinant and that these molecules could be sufficient for the activation of the B lymphocytes. The equivalent susceptibility of homozygous and heterozygous cells seems more consistent with the concept that interaction of anti-Lyb 7.1 antibodies with Lyb 7.1 molecules may deliver an off signal to the cell and thus lead to inhibition of responses of Lyb 7+ B cells. Indeed, Lyb 7 may be a site through which suppression is mediated in vivo and inhibition by anti-Lyb 7.1 may be analogous to a physiological process.

It is interesting to note that an alloanti-δ serum also selectively inhibits responses to TI-2 antigens (28). The characteristics of inhibition by anti-Lyb 7.1 and anti-δ are similar but the action of anti-Lyb 7.1 is not due to its contamination with anti-δ antibody. This is shown both by analysis of membrane proteins precipitated by anti-Lyb 7.1 and by genetic studies which show a segregation of genes for Lyb 7 and δ.²

Finally, anti-Lyb 3, which is similar to anti-Lyb 5 to the extent that it defines an antigen present only on a subset of B lymphocytes, also has some functional properties (30). This antiserum has been shown to substitute for T cell help in vivo under certain conditions. Although anti-Lyb 5 serum could contain anti-Lyb 3-like activity, no functional evidence of such activity was seen in the present in vitro experiments. However, it is formally possible that the activation effects of anti-Lyb 3 and the blocking effects of anti-Lyb 7 simply reflect different consequences of the same interaction, dictated by the precise conditions in which they occur.
Summary

Lyb 5 is a B-cell alloantigen which is expressed on 50–60% of B cells. It was defined originally on the basis of cytotoxicity. We have described a new reactivity within the anti-Lyb 5 serum on the basis of selective inhibition of antibody responses in vitro by this antiserum in the absence of complement. This inhibitory activity of anti-Lyb 5.1 serum appears to be due to recognition of antigenic determinants different from the prototype antigens detected in the cytotoxicity assay.

Anti-Lyb 5 serum incorporated into spleen cell cultures selectively inhibits antibody responses to a class of thymus-independent antigens (TI-2) previously characterized by their failure to elicit antibody formation in immature mice or in the defective CBA/N strain. Responses to optimal concentrations of TI-1 antigens, which can induce antibody synthesis in these mice, are unaffected by the addition of anti-Lyb 5.1 serum. The B-cell alloantigen defined by this functional assay is designated tentatively Lyb 7 and it is shown to be distinct from cell surface immunoglobulins. Lyb 7 appears to have a role in the activation of B lymphocytes by the TI-2 class of thymus-independent antigens.

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