ON THE FUNCTION OF Ly-5 IN THE REGULATION OF ANTIGEN-DRIVEN B CELL DIFFERENTIATION
Comparison and Contrast with Lyb-2*

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The precise mechanisms and fine regulation of antigen-driven B cell activation and differentiation must surely depend on several cell surface molecular systems in addition to immunoglobulin. One way to investigate the reception of regulatory signals by B cells, and the responses that ensue, is to use antibodies to immunogenetically defined components of the B cell surface as probes to perturb one or another feature of B cell responses to various antigens, and thereby infer a particular function for each component.

The components of most evident interest, clearly, are those whose expression is limited to B cells, or to B cells and collaborating cells. In the former category is Lyb-2, an early B cell surface feature lacking on the terminal plaque-forming cells (PFC)¹ (1), which we have shown to be involved at the time of B cell triggering by T-dependent (TD) antigen (2, 3). The Ly-5 system (4) belongs to the latter category, since one or another form of this Ly-5 molecular family (5) is expressed by T cells and macrophages (Mφ) as well as by other hematopoietic cell lineages (6-8). The studies described in the present report signify a distinctive role for Ly-5 in B cell function that differs from that of Lyb-2.

Materials and Methods

Mice. Mice 8-14 wk of age were obtained from colonies at Sloan-Kettering Institute for Cancer Research, except for C57BL/6 (B6) and DBA/2 (purchased from The Jackson Laboratory, Bar Harbor, ME).

Antigens. Sheep erythrocytes (SRBC) were purchased from Gibco Diagnostics Laboratories, Madison, WI. Trinitrophenylated Ficoll (TNP-F) was prepared as described by Inman (9). Aminoethylcarboxymethyl00-F (Biosearch, San Rafael, CA) was conjugated with 2,4,6-trinitrobenzenesulfonic acid (Eastman Kodak Co., Rochester, NY) in 0.2 M borate buffer, pH 9.2, at room temperature for 2 h and then dialyzed extensively against phosphate-buffered saline. This preparation contained 36 TNP groups per 400,000 daltons of F. TNP-Brucella abortus (TNP-BA) was prepared as described (10) using BA ring test antigens (U.S. Department of Agriculture, Ames, IA). TNP-lipopolysaccharide (TNP-LPS) was prepared as described (11) using LPS from Escherichia coli 0111:B4 (Difco Laboratories, Detroit, MI).

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Abbreviations used in this paper: a, anti; BA, Brucella abortus; C, complement; F, Ficoll; LPS, lipopolysaccharide; mc, monoclonal; Mφ, macrophage; MLC, mixed lymphocyte culture; PFC, plaque-forming cells; SN, supernatant; SRBC, sheep erythrocytes; TD, thymus-dependent; TI-1 and TI-2, thymus-independent (antigens) types 1 and 2; TNP, trinitrophenyl.
Monoclonal (mc) Alloantibodies. All studies were done with the mc Ly-5 alloantibodies (ascites plus serum) anti-Ly-5.1 (clone 104-2) and anti-Ly-5.2 (clone A-20) (12). Both are γδκ and critically distinguish the B6 and B6-Ly-5.2 congenic strains. Both react with adherent accessory, T, and B cells in protein A binding and direct immunofluorescence assays. The Lyb-2.1 mc antibody was the same as used in previous studies (1-3).

Cell Preparations

T cells. These were prepared by nylon selection of spleen cells (13).

T-depleted spleen cells. T cells were eliminated by exposure of spleen cells first to mc-α-Thy-1.2 (1:100) plus rabbit serum complement (C) and then to [mc-α-Lyt-1.2 (1:25) and mc-α-Lyt-2.2 (1:25)] plus C as described (3). The two treatments were required to eliminate residual T cells effectively (3).

Mφ. Splenic adherent cells treated with mc-α-Thy-1.2 plus C and irradiated with 1,500 rad were used as Mφ (3).

Mφ-depleted cells. Spleen cells (~18 × 10^7) were passed over a Sephadex G-10 column (bed volume, 30 ml; Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) and eluted with 25 ml of RPMI 1640 (Grand Island Biological Co., Grand Island, NY) containing 20% fetal bovine serum (FBS) and antibiotics (14), and the procedure was repeated. This treatment reduced the α-SRBC PFC response by >95% and esterase-positive cells from 5-9% to <0.5%, without significant disproportion of T and B cells, determined by immunofluorescence with fluorescein-conjugated mc-α-Thy-1.2 and rabbit α-mouse immunoglobulin.

B cells. Mφ-depleted spleen cells were treated as above for elimination of T cells.

Mishell-Dutton Culture. Spleen cells, untreated or as combinations of fractionated cell populations (see above), were cultured in RPMI 1640 supplemented with 10% FBS, l-glutamine (2 mM), 2-mercaptoethanol (5 × 10^-5 M), sodium pyruvate (1 mM), streptomycin (100 µg/ml), penicillin (100 U/ml), and Hepes buffer (10 mM) in 16-mm flat-bottomed Linbro plates (Linbro Chemical Co., Hamden, CT) with SRBC (5 × 10^6), TNP-BA (1:1,000 of stock), TNP-LPS (1 µg/ml), or TNP-F (1 ng/ml), unless otherwise stated, at 37°C in a humidified atmosphere of 10% CO_2 and 90% air without rocking. Cultures were fed daily as in (2).

PFC Assay. PFC counts were determined on day 5 for α-SRBC, and on day 4 for α-TNP responses to T-independent (TI) antigens, by the slide version (15) of Jerne plaque assay. Lightly conjugated TNP-SRBC was prepared as described (16). Results were expressed as mean PFC per culture ± SEM or as a percentage of the standard control response [100 (PFC experiment)/(PFC control)].

Preparation of Factors

Mixed lymphocyte culture supernatant (MLC-SN). 5 × 10^6 B6 spleen cells were stimulated with 5 × 10^6 irradiated DBA/2 spleen cells (2,000 rad) in 1 ml of Mishell-Dutton medium (MDM) in a Linbro well. 24 h later, the SN was centrifuged, filtrated, and stored at -20°C. Higher concentrations of this preparation had significant Mφ-substituting activity as previously reported (3).

Mφ-SN. 2 × 10^6 P388D.1 cells, kindly provided by Dr. Peter Ralph of the Sloan-Kettering Institute for Cancer Research, were cultured in 1 ml of MDM with 10 ng/ml phorbol myristate acetate (Sigma Chemical Co., St. Louis, MO) in a Linbro well for 4 d. The cell-free SN was dialyzed against 100 vol of RPMI 1640, filtrated, and stored at -20°C. This preparation, at concentrations used in this study (0.1-4%), had negligible capacity to replace T cell activity in supporting α-SRBC PFC generation from T-depleted cells.

Results

Inhibition of Generation of α-SRBC PFC by mc Ly-5 Alloantibody (Fig. 1). Spleen cells from B6 (Ly-5.1) and congenic B6-Ly-5.2 mice were cultured with SRBC in the presence of various concentrations of mc-α-Ly-5.1 for 5 d. Fig. 1 shows that mc-α-Ly-5.1 antibody at concentrations of from 1:100 to 1:1,600 inhibited α-SRBC PFC generation from B6 cells by 75-83%. Specificity for Ly-5 is shown by the fact that same antibody caused no significant reduction in the responses from congenic B6-Ly-5.2.
YAKURA, SHEN, BOURCET, AND BOYSE

Fig. 1. Inhibition of generation of \( \alpha \)-SRBC PFC by Ly-5 mc alloantibody. \( 5 \times 10^6 \) B6 or B6-Ly-5.2 (control) spleen cells were cultured with \( 5 \times 10^8 \) SRBC in the presence of mc-\( \alpha \)-Ly-5.1 throughout the 5-d culture period. Control responses (without antibody) were 5,825 \( \pm \) 235 for B6, and 3,952 \( \pm \) 125 for B6-Ly-5.2 (standard 100% response for calculation of percent PFC response).

**Table I**
The Reduction of \( \alpha \)-SRBC PFC Generation by Ly-5 Antibody Is Due To Its Reaction with Non-\( T \) Cells

| Experiment | Composition of culture | Percent response with mc-\( \alpha \)-Ly-5.1 | Control PFC response (no antibody) |
|------------|------------------------|----------------------------------|----------------------------------|
|            | T-depleted cells*      | T cells*                         | 1:400                            | 1:800                            |
| Strain     | Number \( \times 10^8 \) | Strain Number \( \times 10^8 \) |                                   |                                  |
| 1.         | B6                     | 4 B6                             | 1                                | 35 NT§ 6,065 \( \pm \) 790       |
|            | B6-Ly-5.2              | 4 B6-Ly-5.2                      | 1                                | 114 NT 2,547 \( \pm \) 259       |
|            | B6                     | 4 B6-Ly-5.2                      | 1                                | 38 NT 6,125 \( \pm \) 503        |
|            | B6                     | 4 B6-Ly-5.2                      | 0.5                              | 48 NT 2,853 \( \pm \) 569        |
|            | B6-Ly-5.2              | 4 B6                            | 1                                | 85 NT 5,766 \( \pm \) 419        |
|            | B6-Ly-5.2              | 4 B6                            | 0.5                              | 88 NT 3,746 \( \pm \) 122        |
| 2.         | B6                     | 5 B6-Ly-5.2                      | 2                                | 29 30 12,037 \( \pm \) 706       |
|            | B6                     | 5 B6-Ly-5.2                      | 1                                | 33 35 9,709 \( \pm \) 395        |
|            | B6-Ly-5.2              | 5 B6                            | 1                                | 94 97 3,711 \( \pm \) 198        |
|            | B6-Ly-5.2              | 5 B6                            | 1                                | 93 99 3,951 \( \pm \) 676        |

* Responses of T-depleted cells alone, and of T cells alone, were nil.
† 100 (response with antibody)/(response without antibody).
§ Not tested.

5.2 cells (Fig. 1). The same results (not shown) were obtained with mc-\( \alpha \)-Ly-5.2 antibody, with specificity for B6-Ly-5.2 congenic spleen cells.

**Target Cells Affected by Ly-5 Antibody (Tables I and II).** Ly-5 is expressed by most or
The Reduction of PFC Generation by Ly-5 Antibody Is Due To Its Reaction with B Cells rather than Mφ

| Experiment | Composition of culture | Percent response with mc-α-Ly-5.1 | Control PFC response (no antibody) |
|------------|------------------------|-----------------------------------|----------------------------------|
|            | Mφ-depleted cells*     | Mφ*                               |                                  |
|            | Strain Number          | Strain Number                      | 1:500                            |
|            | × 10⁶                  | × 10⁴                              |                                  |
| 1          | B6 4                   | B6 8                              | 32                               |
|            | B6-Ly-5.2 4            | B6-Ly-5.2 8                       | 98                               |
|            | B6 4                   | B6-Ly-5.2 8                       | 24                               |
|            | B6-Ly-5.2 4            | B6-Ly-5.2 8                       | 38                               |
|            | B6 4                   | B6 8                              | 96                               |
|            | B6-Ly-5.2 4            | B6 8                              | 96                               |
| 2          | B6 5                   | B6-Ly-5.2 8                       | 38                               |
|            | B6 5                   | B6-Ly-5.2 8                       | 40                               |
|            | B6-Ly-5.2 5            | B6 8                              | 120                              |
|            | B6-Ly-5.2 5            | B6 8                              | 140                              |

* Responses of Mφ-depleted cells alone: B6, 133 ± 24 (experiment 1) and 380 ± 84 (experiment 2); B6-Ly-5.2, 93 ± 27 (experiment 1) and 281 ± 28 (experiment 2). Responses of Mφ alone were all nil.

| All hematopoietic cell types. To determine whether T and non-T cells are involved in the reduction of α-SRBC PFC generation by Ly-5 antibody (above), spleen cells from B6 and B6-Ly-5.2 mice were fractionated into T and T-depleted populations, combined in all four possible combinations, and cultured with or without mc-α-Ly-5.1 at concentrations of 1:400 and 1:800. As shown in Table I, α-SRBC PFC generation was reduced only when the T-depleted population (B and accessory cells) was Ly-5.1; the Ly-5 type of the T population was irrelevant. This implies that the action of Ly-5 antibody in reducing PFC generation is on B or accessory cells rather than T cells. A similar fourfold combination was then used to determine whether the PFC-reducing effect of Ly-5 antibody is due to its reaction with B cells or with Mφ. The two cell donors, as before, were B6 and B6-Ly-5.2, the cell populations in this case being Mφ depleted and Mφ selected. Table II shows that PFC responses were reduced only when the phenotype of the Mφ-depleted cells was Ly-5.1, corresponding to the antibody; the phenotype of the Mφ was irrelevant. This implies that the cells mostly concerned in PFC reduction by Ly-5 antibody are B cells.

Evidence against Suppression or a Shift of Kinetics (Table III, Fig. 2). To test whether the PFC inhibitory property of Ly-5 antibody might be due to indirect or direct activation of suppressor cells, two sets of experiments were performed. The first experiments tested the possibility that the interaction of B cells with Ly-5 antibody induces Lyt-2⁺ suppressor cells. Nylon-selected splenic T cells were treated with Lyt-2 antibody and C under predetermined optimal conditions for elimination of Lyt-2⁺ cells (removing the Lyt-123 and Lyt-23 cell sets), and were then added to T-depleted spleen cells. This pre-elimination of Lyt-2⁺ cells did not affect the PFC-reducing capacity of Ly-5 antibody (Table III).

In the second set of experiments, unselected spleen cells from B6 and B6-Ly-5.2 donors were combined in serial proportions and cultured with mc-α-Ly-5.1 (1:800) or
Table III

**Lyt-2** Cells Are Not Involved in Reduction of PFC Generation Caused by Ly-5 Antibody

| Spleen cell donor   | T cells added* | Percent PFC response with: |
|---------------------|---------------|----------------------------|
|                     |               | mc-α-Ly-5.1 (1:1,000)      |
| B6                  | Unselected    | 31                         |
|                     | Lyt-1         | 35                         |
| B6-Ly-5.2           | Unselected    | 40                         |
|                     | Lyt-1         | 34                         |

* Nylon-purified T cells were treated either with normal mouse serum (for unselected cells) or with mc-α-Lyt-2.2 antibody (for Lyt-1 cells) plus C. 1 × 10⁸ unselected cells, or 1 × 10⁸ selected Lyt-1 cells, were added to 4 × 10⁸ syngeneic T-depleted cells.

Fig. 2. Evidence against suppression. B6 and B6-Ly-5.2 spleen cells were combined in the proportions indicated and cultured with SRBC in the presence of mc-α-Ly-5.1 (○, 1:800) or mc-α-Ly-5.2 (●, 1:400).

mc-α-Ly-5.2 (1:400). Fig. 2 shows a virtually linear relationship between the degree of PFC reduction and the proportion of cells of Ly-5 type corresponding to the Ly-5 antibody. These data are not compatible with any evident model of suppression. The second set of experiments is valuable in excluding possible suppression by non-T cells (B cells or Mφ).

In the other experiments it was observed that the peak time of PFC generation was same in the presence or absence of Ly-5 antibody, signifying that the lower peak in the presence of antibody is not due to delay or acceleration in the rate of PFC generation.

**Effect of Ly-5 Antibody on PFC Generation to TI Antigens of Types 1 and 2 (Table IV and Fig. 3).** Table IV shows that Ly-5 antibody, at concentrations causing maximal reduction of α-SRBC PFC generation, did not demonstrably inhibit generation of PFC to the two TI-1 antigens tested, TNP-BA and TNP-LPS. This was equally true...
TABLE IV

Effect of Ly-5 Antibody on Responses to TI-1 Antigens

| Antigen  | Spleen cell donor | Antibody concentration | Percent PFC response* |
|----------|------------------|------------------------|-----------------------|
| TNP-BA   | B6               | mc-α-Ly-5.1 1:100      | 153 ± 3               |
| (1:10^5) |                  | 1:200                  | 120 ± 6               |
|          |                  | 1:400                  | 88 ± 1                |
|          |                  | 1:800                  | 92 ± 9                |
|          | B6-Ly-5.2        | mc-α-Ly-5.2 1:100      | 123 ± 3               |
|          |                  | 1:200                  | 97 ± 14               |
|          |                  | 1:400                  | 103 ± 19              |
|          |                  | 1:800                  | 113 ± 19              |
| TNP-LPS  | DBA/2            | mc-α-Ly-5.1 1:100      | 82 ± 14               |
| (1 μg/ml) |                  | 1:400                  | 88 ± 15               |
|          |                  | 1:1,600                | 94 ± 8                |

* Mean PFC responses (± SEM) of two with TNP-BA and five experiments with TNP-LPS.

‡ The full range of antigen concentrations tested, with results that were the same as those illustrated in this Table, were 10^-3 to 10^-4 dilutions of stock for TNP-BA and 0.1 to 100 μg/ml for TNP-LPS (see text).

Fig. 3. Inhibition of PFC response to TNP-F by Ly-5 antibody. (A) Effect of antibody concentration. 10 × 10^6 B6, DBA/2 (Ly-5.1), or B6-Ly-5.2 congenic (control) spleen cells were cultured with TNP-F (1 ng/ml) in the presence of the concentrations of mc-α-Ly-5.1 indicated for 4 d. (B) Effect of antigen concentration. 10 × 10^6 DBA/2 spleen cells were cultured in the presence (○) or absence (□) of mc-α-Ly-5.1 (1:100) with the range of TNP-F antigen concentrations indicated.

for antigen concentrations ranging from 0.1 to 100 μg/ml for TNP-LPS and for 10^-3 to 10^-6 dilutions of stock for TNP-BA (data not shown).

The prototype TI-2 antigen tested, TNP-F, gave a quite different result. This mc-α-Ly-5.1 antibody in concentrations >1:1,600 gave strong Ly-5-specific inhibition of α-TNP PFC (Fig. 3, panel A), and this inhibition was evident with TNP-F antigen concentrations ranging from 10^2 to 10^-4 μg/ml (panel B). The degree of inhibition of α-TNP PFC was similar to that of α-SRBC PFC, roughly 60–80% reduction. DBA/2 was included in these studies as a high responder to the antigens used.

Time of Action of Ly-5 Antibody (Fig. 4). Fig. 4 shows that Ly-5 antibody added >24 h after initiation of culture is relatively ineffective in reducing PFC generation to SRBC and TNP-F, and antibody addition delayed for >48 h has virtually no effect.
In view of the persistent expression of Ly-5 on mature B cells, these data imply that Ly-5 is involved in an early phase of B cell activation that is complete within 2 d.

**Generation of PFC from Ly-5 Heterozygous Cells in the Presence of Ly-5 Antibody (Table V).** The purpose of this test was to find whether Ly-5 antibody might suppress PFC generation by direct effect upon B cells rather than by obstructing function of the Ly-5 molecule. In the case of the Lyb-7 system, antibody similarly suppresses PFC generation to the TI-2 antigen TNP-F, and this suppression affects Lyb-7 heterozygotes as well as homozygotes, which would allow the former interpretation (17). This is not so with Ly-5. Thus mc-α-Ly-5.1 antibody caused no reduction in PFC generation from Ly-5 heterozygous cells with TNP-F or SRBC (Table V).

**Effect of Ly-5 Antibody on α-SRBC PFC Generation Assisted by T and Mφ Factors (Table VI and Figs. 5, 6, and 7).** Generation of PFC to SRBC and to TNP-F depends on both T cells and Mφ (18–21). The question arises whether Ly-5 is involved in the B cell response to T or Mφ signals. The rationale of the following experiments is that if Ly-5 is concerned in the interaction of B cells with T cells or Mφ, then PFC reduction by Ly-5 antibody should be competitively overcome by T or Mφ helper factors.

**Responses of T-depleted cells assisted by MLC-SN.** Table VI illustrates that Ly-5 antibody caused maximal α-SRBC PFC reduction in concentrations of MLC-SN as high as 40%. Even in 80% MLC-SN, reduction was only slightly less, and this small effect could well be due to minor contamination of MLC-SN with Mφ factor.
TABLE VI
Reduction of α-SRBC PFC Generation from T-depleted Cells by Ly-5 and
Lyb-2 Antibodies in the Presence of MLC-SN*

| Concentration of MLC-SN | Percent response with | Control response (no antibody) |
|------------------------|----------------------|-------------------------------|
|                        | mc-α-Ly-5.1 | mc-α-Lyb-2.1                     |                                |
| 10%                   | 35         | 32                               | 1,284 ± 153                   |
| 20%                   | 25         | 29                               | 7,043 ± 521                   |
| 40%                   | 30         | 18                               | 23,129 ± 5283                 |
| 80%                   | 50         | 5                                | 116,615 ± 19041               |

* One of three similar experiments is shown. 5 × 10⁶ T-depleted cells from B6-Lyb-2.1 mice were cultured with SRBC and serial concentrations of MLC-SN in the presence of mc-α-Ly-5.1 (1:100) or mc-α-Lyb-2.1 (1:50). α-SRBC PFC were assayed on day 5.

Evidently MLC-SN does not competitively inhibit the action of Ly-5 antibody. In parallel tests with mc Lyb-2 antibody, to represent a system known to be concerned in early B cell activation and evidently not through the T cell interaction process, MLC-SN did not competitively inhibit the action of Lyb-2 antibody in reducing PFC generation either (Table VI).

Fig. 5. With Mφ-depleted cells, Mφ-SN competitively inhibits PFC reduction by Ly-5 but not Lyb-2 antibody. 4 × 10⁶ Mφ-depleted cells from B6-Lyb-2.1 congenic mice were cultured with serial concentrations of Mφ-SN in the absence (○) or presence of mc-α-Ly-5.1 (●, 1:100) or mc-α-Lyb-2.1 (▲, 1:50) antibody. Representative data for one of three experiments.

Responses of Mφ-depleted cells assisted by Mφ-SN (Fig. 5). To test whether Ly-5 might be concerned in the process of B cell interaction with Mφ, e.g., as a receptor for Mφ signals, the effect of Ly-5 antibody was assessed on responses of Mφ-depleted cells (composed of T and B cells) assisted by 0.5-4% Mφ-SN. If Ly-5 is involved in the process of Mφ signal reception, Mφ-SN should compete with Ly-5 antibody at some
YAKURA, SHEN, BOURCET, AND BOYSE

FIG. 6. With purified B cells and MLC-SN, Mφ-SN competitively inhibits PFC reduction by Ly-5 but not Lyb-2 antibody. Purified B cells (4 × 10⁶) from B6-Lyb-2.1 congenic mice were cultured with the serial concentrations of Mφ-SN shown, together with mc-α-Ly-5.1 (●, 1:100) or mc-α-Lyb-2.1 (▲, 1:50) antibody. On day 2, 15% MLC-SN was added to provide T helper factors, and PFC were assayed on day 5. Mean data from three experiments. Responses of B cells plus MLC-SN without antibody or Mφ-SN (not shown) were <30% of control response with 0.125% Mφ-SN and <15% of control responses with 0.25 to 4% Mφ-SN. Responses of B cells with Mφ-SN alone, at all concentrations, were always <3% of controls, which included MLC-SN.

RESPONSES OF PURIFIED B CELLS ASSISTED BY Mφ-SN AND MLC-SN (FIGS. 6 AND 7). The following experiments confirm that Ly-5 is concerned in the interaction of B cells with Mφ. Purified B cells from B6-Lyb-2.1 congenic mice (Ly-5.1, Lyb-2.1) were cultured with a wide range of Mφ-SN (0.125–4%) in the presence of mc-α-Ly-5.1 or
mc-α-Lyb-2.1 antibody. As a source of T helper factors, 15% MLC-SN was added 2 d later. As shown in Fig. 6, Ly-5 antibody strongly inhibited PFC generation from B cells cultured with lower concentrations of Mϕ-SN (0.125–0.5%), but this blocking effect was progressively abrogated at higher concentrations (2–4%). Thus the PFC-reducing effect of Ly-5 antibody was reversed by overloading with Mϕ-SN in dose-dependent fashion. In contrast, Lyb-2 antibody strongly inhibited PFC generation from B cells assisted by Mϕ-SN irrespective of Mϕ-SN concentrations.

If Ly-5 is concerned in reception or operation of Mϕ factor, then PFC inhibition by Ly-5 antibody should be maximal when antibody is added at the time of action of Mϕ factor, and this is so. When Mϕ-SN was added to B cell cultures at the time of initiation, Ly-5 antibody was effective only when added at that time (Fig. 7, panel A). When addition of Mϕ-SN was delayed for 44 h, Ly-5 antibody was effective when added up to 44 h later, but not thereafter (panel B). The contrast with Lyb-2 is evident in panels A and B.

These results imply that Ly-5 mediates a B cell activation process that is distinct from the action of Lyb-2 and that Ly-5 antibody either reacts with a molecule of high affinity for helper factors present in Mϕ-SN but not for T helper factors present in MLC-SN, or affects this process by inactivating signals generated by the reaction of Mϕ factor with its receptor.

**Discussion**

These studies of the immune functional correlates of Ly-5 followed the lines that were adopted for previous study of Lyb-2. Unlike Lyb-2, expression of Ly-5 is not confined to B cells but occurs also in T cells, Mϕ, and other hematopoietic lineages. Nevertheless the only functions we have seen to be significantly affected by inclusion of Ly-5 antibody in assay of immune function in vitro are those associated with B cells. Since T cells and Mϕ have functions other than immunological—T cells are concerned in erythropoiesis (22–24), for example—Ly-5 on non-B cells might have nonimmunological functions that have not been studied.

In comparing the action of Lyb-2 and Ly-5 mc antibodies in immune functional assays, the points of factual similarity are that both inhibit the generation of PFC to TD antigen (SRBC) but not to TI-1 antigen (TNP-LPS and TNP-BA), and in neither case can this be attributed to suppression by direct action upon the B cell or indirect action through other cells. A critical distinction between the two systems is that Ly-5 antibody inhibits PFC generation to TI-2 antigen (TNP-F), whereas Lyb-2 antibody does not. The salient property of TNP-F in this connection is that with this antigen, like SRBC, the PFC response in vitro depends on T cells and Mϕ (18–21), in contrast to TI-1 antigen. On these grounds Ly-5 could be involved in reception or effects of T cell or Mϕ signals, or alternatively Ly-5 could be concerned in early B cell triggering to TD and TI-2 antigen. However, the former interpretation is strongly favored by the finding that Mϕ factor competitively inhibits the action of Ly-5 antibody in reducing PFC generation to SRBC, and that PFC inhibition by Ly-5 antibody appears to be inseparable from the time of action of Mϕ factor.

**Summary**

Generation of anti-sheep erythrocyte plaque-forming cells (PFC) is greatly reduced in the presence of monoclonal Ly-5 alloantibody. Although Ly-5 is expressed in one
of its molecular forms on T cells and macrophages (Mφ) involved in this response, the 
only demonstrated action of Lyt-5 antibody was on B cells. Evidence from elimination 
of Lyt-2⁺ cells, and from the responses of serial proportions of Lyt-5.1 and Lyt-5.2 cells 
and of Lyt-5 heterozygous cells, signifies that PFC reduction cannot be ascribed to any 
known mechanism of suppression or to a direct suppressive action of Lyt-5 antibody 
on B cells. A critical distinction of Lyt-5 from Lyt-2 is that Lyt-5 antibody reduces 
PFC generation to trinitrophenylated Ficoll, a thymus-independent type 2 antigen 
requiring T cells and Mφ for maximal PFC generation in vitro. A second distinction 
is that PFC reduction by Lyt-5 antibody is strictly tied to the time of operation of Mφ 
factor, whereas PFC reduction by Lyt-2 antibody relates to the time of B cell 
triggering by antigen. Accordingly, Mφ factor competitively and quantitatively 
inhibits the action of Lyt-5 antibody in reducing PFC generation. It is likely that the 
Lyt-5 system is concerned in the reception or handling of Mφ message by B cells.

References
1. Yakura, H., F.-W. Shen, E. A. Boyse, and L. Tang. 1980. The Lyb-2 phenotype of 
hemolytic PFC. Immunogenetics. 10:603.
2. Yakura, H., F.-W. Shen, M. Kaemmer, and E. A. Boyse. 1981. Lyb-2 system of mouse B 
cells. Evidence for a role in the generation of antibody-forming cells. J. Exp. Med. 153:129.
3. Yakura, H., F.-W. Shen, E. Bourcet, and E. A. Boyse. 1982. Evidence that Lyt-2 is critical 
to specific activation of B cells before they become responsive to T and other signals. J. 
Exp. Med. 155:1309.
4. Komuro, K., K. Itakura, E. A. Boyse, and M. John. 1975. Lyt-5: a new T lymphocyte 
antigen system. Immunogenetics. 1:452.
5. Michaelson, J., M. P. Scheid, and E. A. Boyse. 1979. Biochemical features of Lyt-5 
alloantigen. Immunogenetics. 9:193.
6. Scheid, M. P., and D. Triglia. 1979. Further description of the Lyt-5 system. Immunogenetics. 
9:423.
7. Cantor, H., M. Kasai, F.-W. Shen, J. C. Leclerc, and L. Glimcher. 1979. Immunogenetic 
analysis of "natural killer" activity in the mouse. Immunol. Rev. 44:3.
8. Tung, J. S., M. P. Scheid, M. A. Pierotti, U. Hämmerling, and E. A. Boyse. 1981. 
Structural features and selective expression of three Lyt-5⁺ cell surface molecules. Immuno-
genetics. 14:101.
9. Inman, J. K. 1975. Thymus-independent antigens: the preparation of covalent hapten-
Ficoll conjugates. J. Immunol. 114:704.
10. Mond, J. J., I. Scher, D. E. Mosier, M. Bease, and W. E. Paul. 1978. T-independent 
responses in B cell-defective CBA/N mice to Brucella abortus and to trinitrophenyl (TNP) 
conjugates of Brucella abortus. Eur. J. Immunol. 8:459.
11. Jacobs, D. M., and D. C. Morrison. 1975. Stimulation of a T-independent primary anti-
hapten response in vitro by TNP-lipopolysaccharide (TNP-LPS). J. Immunol. 114:360.
12. Shen, F.-W. 1981. Monoclonal antibodies to mouse lymphocyte differentiation alloantigens. 
In Monoclonal Antibodies and T-Cell Hybridomas. Perspectives and Technical Advances. 
G. J. Hämmerling, U. Hämmerling, and J. F. Kearney, editors. Elsevier/North-Holland 
Biomedical Press, Amsterdam. 25-31.
13. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation 
of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3:645.
14. Ly, I. A., and R. I. Mishell. 1974. Separation of mouse spleen cells by passage through 
columns of sephadex G-10. J. Immunol. Methods. 5:239.
15. Cunningham, A. J., and A. Szendreg. 1968. Further improvements in the plaque technique 
for detecting single antibody-forming cells. Immunology. 14:599.
16. Rittenberg, M., and K. Pratt. 1969. Anti-trinitrophenyl (TNP) plaque assay. Primary response of BALB/c mice to soluble and particulate immunogen. *Proc. Soc. Exp. Biol. Med.* 32:575.

17. Subbarao, B., D. E. Mosier, A. Ahmed, J. J. Mond, I. Scher, and W. E. Paul. 1979. Role of a nonimmunoglobulin cell surface determinant in the activation of B lymphocytes by thymus-independent antigens. *J. Exp. Med.* 149:495.

18. Chused, T. M., S. S. Kassan, and D. E. Mosier. 1976. Macrophage requirement for the in vitro responses to TNP Ficoll: a thymus independent antigen. *J. Exp. Med.* 149:495.

19. Boswell, H. S., S. O. Sharrow, and A. Singer. 1980. Role of accessory cells in B cell activation. I. Macrophage presentation of TNP-Ficoll: evidence for macrophage-B cell interaction. *J. Immunol.* 124:989.

20. Mond, J. J., P. K. A. Mongini, D. Sieckman, and W. E. Paul. 1980. Role of T lymphocytes in the response to TNP-AECM-Ficoll. *J. Immunol.* 125:1066.

21. Letvin, N. L., B. Benacerraf, and R. N. Germain. 1981. B-lymphocyte responses to trinitrophenyl-conjugated Ficoll: requirement for T lymphocytes and Ia-bearing adherent cells. *Proc. Natl. Acad. Sci. USA.* 78:5113.

22. Wiktor-Jedrzejczak, W., S. Sharkis, A. Ahmed, K. W. Sell, and G. W. Santos. 1977. Thetasensitive cell and erythropoiesis: identification of a defect in W/Wv anemic mice. *Science (Wash. DC).* 196:313.

23. Goodman, J. W., N. L. Basford, and S. G. Shinpock. 1978. On the role of thymus in hemopoietic differentiation. *Blood Cells.* 4:53.

24. Torok-Storb, B., and J. A. Hansen. 1982. Modulation of *in vitro* BFU-E growth by normal Ia-positive T cells is restricted by HLA-DR. *Nature (Lond.)*. 298:473.