Specific antigen binding by thymus-derived (T) and bone marrow-derived (B) cells has been demonstrated in both humoral (1-5) and cell-mediated (6-8) immune responses. The receptor for antigen on mature antibody-forming cells (AFC) and AFC precursors (both are B cells) has been clearly shown to be an immunoglobulin molecule (1, 3, 9-11). Evidence has accumulated that the antigen receptor on “helper” cells (2, 4, 12, 13, 5) and cytotoxic lymphocytes (4, 5, 12-14) (both are T cells) is also an immunoglobulin. However, T cells appear to have far fewer antigen receptors than B cells (3, 12, 13). Therefore, it has been possible to distinguish these two cell types by their differences in antigen-binding ability (3, 15, 16).

Since both T cells and B cells can bind sheep red blood cells (SRBC) specifically to form rosettes (9, 17, 18, 19), a technique was developed to characterize T rosette-forming cells (RFC) and B RFC by their SRBC-binding ability (15, 16). All T RFC bound less than 10 SRBC, whereas almost all B RFC bound at least 10 SRBC. With the technique of velocity sedimentation at unit gravity (20, 21), RFC of different SRBC-binding ability could be purified for functional studies (16). B RFC binding 10-18 SRBC (designated B1 RFC) were shown to correlate with plaque-forming cell (PFC) precursors; RFC binding greater than 18 SRBC (designated B2 RFC) were shown to correlate with PFC. B-cell function was thus directly linked with RFC binding 10 or more SRBC.

A direct demonstration of the function of T RFC is more difficult because T RFC dissociate within 10 min of being resuspended from the pellet (16). T rosettes, therefore, may be undetected if dissociation is not prevented. Indeed, the instability of T RFC may account for the failure of some workers to demonstrate T RFC in populations of “educated” thymus cells (22). If a metabolic inhibitor, sodium azide, was added to the suspension of rosettes, T RFC did not dissociate (16). Since the effect of azide could be reversed by washing in...
azide-free medium (23), it was possible to stabilize and purify viable T RFC for functional studies. The present study is an evaluation of whether T RFC evidence helper cell activity.

Materials and Methods

Animals.—Female DBA/2 mice (Jackson Laboratory, Bar Harbor, Maine) 8–12 wk of age were used.

Medium.—For all living tissue, Eagle's minimal essential medium (MEM) (Grand Island Biological Co., Grand Island, N. Y.) buffered to pH 7.4 with tris(hydroxymethyl)aminomethane (Trizma base: Gibco) was used. (The following volumes per liter were added: 2.19 M NaCl, 9.6 ml; 10 times isotonic Trizma base, 10 ml.)

Sodium Azide.—A 1% (wt/vol) solution of sodium azide (Fisher Scientific Co., Pittsburgh, Pa.) in phosphate-buffered saline was used in some of the experiments described.

Neuraminidase Treatment.—SRBC (1 × 10^7) were incubated in a solution of neuraminidase (General Biochemicals Div., Chagrin Falls, Ohio.) (50 U/ml in phosphate-buffered saline, pH 7.4) for 30 min at 37°C. The cells were washed once in MEM and 1 × 10^6 spleen cells were added for rosette formation.

Antigens and Immunization.—SRBC obtained from a single donor animal were stored in Alsever's solution at 4°C for at least 1 wk, and then washed three times in MEM before use.

Preparation of Cell Suspensions and Formation of Rosettes.—Cells from normal spleen were teased into MEM. Cell clumps were dispersed by firmly pipetting the suspension with a small bore Pasteur pipette. Remaining cell aggregates were allowed to settle. The single cells in the upper two-thirds of the suspension were removed, washed three times in MEM, and the nucleated cell count determined.

Rosettes were made as reported previously (16) but with the following modifications: 80 × 10^6 nucleated cells and 8 × 10^8 SRBC were resuspended in 2 ml of MEM. In the experiments shown in Fig. 1, Fig. 2, and Fig. 3, SRBC were pretreated with neuraminidase before rosette formation. The cell suspension was centrifuged at 4°C in a 10-ml plastic centrifuge tube (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) at 100 g for 6 min and then incubated at 4°C for 1 h. In some experiments (indicated below) 0.1 ml of 1% sodium azide was added before resuspending the pellet.

Fractionation of Rosettes.—Velocity sedimentation at unit gravity (20) was employed to separate rosettes according to size as previously described (16). Glass sedimentation chambers (22 cm in diameter) (O. H. Johns Scientific, Toronto) were used. The cells were loaded into these chambers in 100 ml of 0.17% Ficoll in MEM. A shallow gradient of 0.35–1.5% Ficoll in MEM was then introduced to lift the cells to the top of the chamber.

Rosettes were formed and without fixation were subjected to velocity sedimentation. In some experiments (indicated below) 0.08% sodium azide was present in the sedimentation medium. After a sedimentation time of 1.5 h 40-ml fractions were collected. A 0.5-ml portion was removed from each fraction before washing for counting rosettes.

Adoptive Transfer of Fractionated Cells.—Pooled fractions of nonrosettes, T rosettes, and B rosettes were injected intravenously in various combinations (indicated below) into 650-R-irradiated syngeneic hosts. 5 × 10^8 SRBC were injected per animal. Controls consisted of irradiated mice injected with SRBC alone, or with spleen cells equivalent to the number of B rosettes injected per animal. Spleens were assayed 7 days later for direct hemolytic PFC by the technique of Cunningham and Szenberg (24).

RESULTS

Relationship between Sedimentation Velocity and Number of SRBC per RFC.—First the relationship between sedimentation velocity and number of SRBC...
Rosettes made from normal spleen were subjected to velocity sedimentation in either the presence or absence of 0.05% azide, and each fraction was reassayed for rosettes. When rosettes were sedimented in azide-free medium, RFC binding less than 10 SRBC dissociated and remained as single cells in the small lymphocyte cell band (Fig. 1). However, when 0.05% azide was present in the sedimentation medium, RFC binding less than 10 SRBC did not dissociate, but retained their rosette morphology and shifted into a higher velocity range. In both experiments RFC velocity was proportional to the number of SRBC bound and when azide was present both T and B rosettes could be separated from small lymphocytes.

Effect of Sodium Azide on Activity of Adoptively Transferred Cells.—In order to show that the inhibition by azide of metabolic function could be reversed by

![Diagram](image)

Fig. 1. Rosettes were made from normal spleen and subjected to velocity sedimentation in either the presence or absence of 0.05% sodium azide. Rosettes were remade on each fraction and the number of SRBC per RFC was determined. Each point represents the average antigen-binding ability of at least 15 rosettes.

washing in azide-free medium, normal spleen cells were incubated at 4°C in the presence or absence of 0.05% sodium azide for 2 h. The cells were then washed three times in azide-free medium. $8 \times 10^6$ nucleated cells were transferred with $5 \times 10^8$ SRBC into 650-R-irradiated hosts. 7 days later, 19S PFC were assayed. The results in Table I indicate that pretreatment with sodium azide had no effect on the PFC response. Therefore, the inhibitory effect of sodium azide can be reversed by washing three times in azide-free medium.

Separation of T and B Rosettes.—When SRBC were treated with neuraminidase before rosette formation the SRBC binding of B RFC increased without altering that of T RFC (16). This enabled a cleaner separation between T and B rosettes by velocity sedimentation as illustrated in Fig. 2a. Here, in the presence of azide, nonrosette small lymphocytes sedimented less than 4.5 mm per h (fraction A). "Blast" cells and lymphocytes binding two or three erythrocytes
THYMUS-DERIVED ROSETTES ARE NOT "HELPER" CELLS

TABLE I
Effect of Azide on 19S PFC Response of Transferred Spleen Cells

| Cells injected          | PFC per spleen |
|------------------------|----------------|
| Spleen – azide         | 8 × 10⁵         | 360 ± 45     |
| Spleen + azide         | 8 × 10⁵         | 440 ± 96     |
| SRBC                   |                | 10 ± 7       |

Spleen cells were incubated at 4°C for 2 h in the presence or absence of 0.05% sodium azide, washed three times in azide-free medium, and injected into 650-R-irradiated animals. Direct PFC per spleen were assayed 7 days later.

Sedimented between 4.5 and 6.5 mm per h. T rosettes binding 4–10 SRBC sedimented between 6.5 and 11.5 mm per h (fractions C and D). Single-layered (B₁) rosettes sedimented between 11.5 and 14.9 mm per h (fraction E). Multilayered (B₂) rosettes sedimented at velocities greater than 14.9 mm per h (fraction F).

Activity of Helper Cells and Precursors of PFC.—An experiment was designed to test rosette fractions of varying sedimentation velocities for the presence of helper cell function or PFC precursor cell function. Each fraction, from normal spleen, was transferred with 5 × 10⁸ SRBC and either 10 × 10⁶ bone marrow cells or 25 × 10⁶ thymus cells into irradiated animals. Direct PFC per spleen were assayed 7 days later. Synergism with bone marrow cells represents helper T-cell activity; synergism with thymus cells represents PFC precursor activity.

The results in Fig. 2 b show that most of the helper cell activity remained in the nonrosette fraction. There was no helper cell activity associated with T rosettes (fractions C and D). Controls, consisting of thymus cells or bone marrow cells with SRBC, or SRBC injected alone, yielded low background PFC values. Fractions A–D injected with thymus cells did not result in any significant PFC response compared to the controls. Bone marrow cells (1.0 × 10⁷) and thymus cells (2.5 × 10⁶) injected together yielded 250 PFC per spleen.

Significant synergism with thymus cells occurred in fraction E where single-layered B₁ RFC sedimented. This fraction therefore contained the majority of PFC precursors. Fraction F which contained multilayered rosettes did not synergize with either bone marrow or thymus cells. This result agrees with the previous report that PFC precursors form single-layered rosettes (B₁ RFC) (16).

Are Helper Cells Very Low Antigen-Binding Cells?—The possibility exists that helper cells bind fewer than four SRBC (which was the lower limit of reliable morphological assessment) and may not have been detected by the rosette technique used (15, 16). Therefore, each rosette fraction from the sedimentation was examined for lymphocytes binding two and three SRBC. The result in Fig. 3 shows that lymphocytes binding two or three SRBC sediment at velocities greater than 4.5 mm per h. Therefore as helper activity was asso-
Fig. 2. (a) Rosettes against neuraminidase-treated SRBC were made from normal spleen cells and sedimented in the presence of sodium azide. Single cells (Δ--Δ) and rosettes (●—●) were counted in each fraction. The morphology of rosettes in each fraction is indicated schematically. (b) Fractions A, B, C, and D were injected with either 1 × 10⁷ bone marrow cells or 2.5 × 10⁷ thymus cells into 650-R-irradiated animals. Direct PFC per spleen were assayed after 7 days. Standard errors are indicated (lines).

Associated with velocities of 4.5 mm per h and less, most of the helper cell activity in Fig. 2b has been correlated with small lymphocytes that do not bind antigen.

Additional Tests.—The results in Fig. 2 are an example of numerous experiments in which combinations of nonrosettes, T rosettes, and B rosettes sep-
Fig. 3. Rosettes were made from normal spleen and subjected to velocity sedimentation in the presence of 0.05% sodium azide. Each fraction was assayed for lymphocytes binding zero, two, or three SRBC.

Fractionated by velocity sedimentation were injected in different combinations into irradiated recipients. Helper T-cell function in both normal thymus cells and normal spleen cells was always associated with the nonrosette fraction. Regardless of whether the source of B RFC was from bone marrow cells or from normal spleen cells, helper cell activity invariably correlated with lymphocytes that did not bind detectable numbers of SRBC.

Analogous studies in vitro have yielded similar results. Nonrosettes and rosettes (both T and B RFC), were isolated by velocity sedimentation with 0.05% sodium azide present. When T and B RFC were cultured together with irradiated (1000 R) syngeneic spleen cells as feeder cells, no 19S PFC response occurred. Only after the nonrosette fraction was added back to the rosette fraction was an anti-SRBC PFC response elicited (J. S. Haskill, unpublished results). These results further indicate that helper T cells in the 19S PFC response do not form rosettes.

**DISCUSSION**

The antigen-binding characteristics of T RFC are different from those of B RFC. Not only do T RFC bind less antigen than B RFC (3, 15, 16), but T RFC also form less stable rosettes which tend to dissociate after being resuspended from the pellet (16). The dissociation of T rosettes may be caused by "capping" of immunoglobulin receptors (18, 25). The stabilization of T RFC with azide (16) is consistent with the hypothesis that there is a rapid turnover of the immunoglobulin receptor for antigen on T lymphocytes (N. L. Warner, personal communication).
With sodium azide present in the suspending medium, a reliable separation of T RFC from nonrosette small lymphocytes could be achieved by velocity sedimentation. When neuraminidase-treated SRBC were used to make rosettes, B RFC bound greater numbers of SRBC while the antigen-binding ability of T RFC was unaffected (16). A clearer separation between T RFC and B RFC could thus be obtained. This separation procedure was a useful tool for obtaining enriched populations of T RFC and B RFC, and for depleting spleen cells of SRBC-specific antigen-binding lymphocytes.

The results in Fig. 2 indicate that T RFC from unprimed animals do not function as helper cells involved in the direct PFC response of bone marrow B cells against SRBC. Other workers have failed to observe increased levels of T RFC (sensitive to anti-θ serum plus complement) in primed animals with elevated helper cell activity (26). Furthermore, Wigzell has failed to bind helper cells on antigen-coated glass bead columns (3, 27). The results now reported are the first direct demonstration that T RFC present in normal spleen are not helper cells.

Although T cells have a wider range of specificities than B cells (28, 29), nevertheless, helper T cells do recognize antigen specifically. Cheers et al. (4) showed that carrier-primed T cells could enhance the antibody response only against haptens coupled onto the carrier used for priming. Feldman has demonstrated an antigen-specific factor secreted by activated T cells in vitro (30). The most convincing evidence for specific antigen recognition is that the helper cell function of unprimed T cells (2) or carrier-primed T cells (5) can be specifically inhibited by “hot antigen suicide.” The results of such experiments indicate that there are receptors for antigen on both unprimed and activated helper T cells, but despite this, such receptors appear not to confer rosette-forming ability to these cells or to result in the retention of these cells on antigen-coated glass bead columns (3, 27). It has been postulated that the receptor for antigen on T cells is buried further below the cell surface than the receptor on B cells (31). This altered configuration might weaken the ability of T cells to bind particulate antigens such as SRBC.

The possibility that T rosettes are completely nonspecific entities produced by cytophilic antibody is unlikely. Lee and Paraskevas (32) found very few T lymphocytes carrying cytophilic antibody in unprimed spleen cells; only after immunization in vivo (32) or after treatment with antigen and adjuvant in vitro (33) could significant numbers of T cells with passively absorbed antibody be detected. T cells in unimmunized animals displayed no detectable cytophilic antibody. Furthermore, when rosettes were made in the presence of two unrelated antigens, SRBC and human RBC, rosettes binding both antigens simultaneously were not formed (B. E. Elliott, unpublished result). Therefore T RFC appear to bind antigen specifically.

The present system has failed to correlate T RFC with helper cell function in the 19S response. Several possibilities remain concerning the physiologic
significance of T RFC. One is that T RFC might represent a later derivative of the helper cell. Whereas functional helper T cells do not bind SRBC, they may mature into T cells which have lost helper function but have developed more receptors (or increased affinity) for antigen and thus the incidental ability to form rosettes. A correlation between maturity and increasing antigen-binding ability is at least characteristic of B-type immunocompetent cells. Early embryonic liver cells contain PFC precursors with very weak antigen-binding properties (B. E. Elliott and D. K. MacFadden, unpublished results). However, immunocompetent cells in animals previously exposed to antigen have greater antigen-binding ability than those in normal spleen (34). Thus PFC precursors develop a greater affinity for antigen as they mature.

Another possibility is that T RFC may represent helper cells in the 7S PFC response. Experiments of Basten et al. (2) provide some support for this hypothesis. These experiments involved a hot antigen suicide technique which resulted in selective inhibition of helper cell activity in the 7S rather than the 19S PFC response (2). T RFC may prove to be helper cells for the 7S PFC response.

A plausible alternative is that T RFC may be lymphocytes associated with the cell-mediated reaction against target SRBC. Several investigators have provided support for this contention. Increased numbers of thymus-derived rosette-forming lymphocytes were found in mice immunized with poly-(A:U) (35). The latter is an adjuvant known to enhance the capacity of an animal to mediate delayed hypersensitivity (35). Increased numbers of T RFC against allogeneic (DBA/2) erythrocytes were found in the peripheral blood of CBA mice which were rejecting a DBA/2 skin graft (36). T RFC might therefore be cells associated with delayed hypersensitivity or xenogeneic graft rejection.

Preliminary experiments have failed to correlate T rosettes in the lymph nodes of mice with effector cells in delayed-type hypersensitivity. The possibility that T RFC are precursors of cells involved in delayed hypersensitivity is being investigated.

In conclusion, there is little doubt that T RFC are thymus-derived antigen-binding cells. The present work is the first in which it has been possible to perform functional tests on an isolated population of T RFC with specificity for a known antigen. In this system it has been shown that T RFC are not the agents of thymic helper activity for the direct PFC response to SRBC. The question of what physiological role is played by T RFC has thus been narrowed but remains open.

SUMMARY

Rosettes against SRBC were made from normal spleen cells. Although T rosettes tend to dissociate, they could be stabilized with 0.05% sodium azide.

Elliott, B. E., and J. S. Haskill. Manuscript in preparation.
A clear separation of nonrosettes, T rosettes, and B rosettes was obtained by subjecting the suspension of splenic rosettes to velocity sedimentation at unit gravity. Each fraction was injected with either normal bone marrow cells or normal thymus cells with antigen into 650-R-irradiated hosts. Direct plaque-forming cells (PFC) were assayed in the spleens 7 days later. Synergism with thymus cells occurred only in the B-rosette fraction; PFC precursors therefore sedimented as B rosettes. Synergism with bone marrow cells occurred only in the nonrosette small lymphocyte fraction; helper cells therefore did not bind detectable numbers of sheep red blood cells (SRBC). Thus T rosettes are not helper cells in the direct PFC response of bone marrow B cells to SRBC.

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REFERENCES

1. Ada, G. 1970. Antigen binding cells in tolerance and immunity. Transplant. Rev. 5:105.
2. Basten, A., J. F. A. P. Miller, N. L. Warner, and J. Pye. 1971. Specific inactivation of thymus-derived (T) and non-thymus-derived (B) lymphocytes by 131I-labelled antigen. Nat. New Biol. 231:104.
3. Wigzell, H. 1970. Specific fractionation of immunocompetent cells. Transplant. Rev. 5:76.
4. Cheers, C., J. C. S. Breitner, M. Little, and J. F. A. P. Miller. 1971. Cooperation between carrier-reactive and hapten-sensitive cells in vitro. Nat. New Biol. 232:248.
5. Roelants, G. E., and B. A. Askonas. 1971. Cell cooperation in antibody induction. The susceptibility of helper cells to specific lethal radioactive antigen. Eur. J. Immunol. 1:151.
6. Wekerle, H., P. Lonai, and M. Feldman. 1972. Fractionation of antigen-reactive cells on a cellular immunosorbent: factors determining recognition of antigens by T-lymphocytes. Proc. Natl. Acad. Sci. U. S. A. 69:1620.
7. Lonai, P., H. Wekerle, and M. Feldman. 1972. Fractionation of specific antigen-reactive cells in an in vitro system of cell mediated immunity. Nat. New Biol. 235:235.
8. Golstein, P., H. Wigzell, H. Blomgren, and E. A. J. Svedmyr. 1972. Cells mediating specific in vitro cytotoxicity. II. Probable autonomy of thymus-processed lymphocytes (T cells) for the killing of allogeneic target cells. J. Exp. Med. 135:890.
9. Greaves, M. F. 1970. Biological effects of antiimmunoglobulins: evidence for immunoglobulin receptors on "T" and "B" lymphocytes. Transplant. Rev. 5:45.
10. Rabellino, E., S. Colon, H. W. Grey, and E. R. Unanue. 1971. Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. J. Exp. Med. 133:156.
11. Herzenberg, L. A., L. A. Herzenberg, R. C. Goodlin, and E. C. Rivera. 1967.
Immunoglobulin synthesis in mice. Suppression by anti-allotype antibody. *J. Exp. Med.* **126:**701.

12. Bankhurst, A. D., N. L. Warner, and J. Sprent. 1971. Surface immunoglobulins on thymus and thymus-derived lymphoid cells. *J. Exp. Med.* **134:**1005.

13. Nossal, G. J. V., N. L. Warner, H. Lewis, and J. Sprent. 1972. Quantitative features of a sandwich radioimmunolabeling technique for lymphocyte surface receptors. *J. Exp. Med.* **135:**405.

14. Cone, R. E., J. Sprent, and J. J. Marchalonis. 1972. Antigen-binding specificity of isolated cell-surface immunoglobulin from thymus cells activated to histocompatibility antigens. *Proc. Natl. Acad. Sci. U. S. A.* **69:**2556.

15. Haskill, J. S., B. E. Elliott, R. S. Kerbel, M. A. Axelrad, and D. Eidinger. 1972. Classification of thymus-derived and bone marrow-derived lymphocytes by demonstration of their antigen-binding characteristics. *J. Exp. Med.* **135:**1410.

16. Elliott, B. E., and J. S. Haskill. 1973. Characteristics of thymus-derived and bone marrow-derived rosette forming lymphocytes. *Eur. J. Immunol.* **3:**68.

17. Bach, J.-F., M. Dardenne, and A. J. S. Davies. 1971. Early effect of adult thymectomy. *Nat. New Biol.* **231:**110.

18. Ashman, R. F., and M. C. Raff. 1973. Direct demonstration of theta-positive antigen-binding cells, with antigen-induced movement of thymus-dependent cell receptors. *J. Exp. Med.* **137:**69.

19. Bach, J.-F., and M. Dardenne. 1973. Antigen recognition by T lymphocytes. III. Evidence for two populations of thymus dependent rosette-forming cells in spleen and lymph nodes. *Cell. Immunol.* **6:**394.

20. Miller, R. G., and R. A. Phillips. 1969. Separation of cells by velocity sedimentation. *J. Cell. Physiol.* **73:**191.

21. Osoba, D. 1970. Some physical and radiobiological properties of immunologically reactive mouse spleen cells. *J. Exp. Med.* **132:**368.

22. Hunter, P., A. Munro, and I. McConnell. 1972. Role of educated T cells in cooperation with B cells. *Nat. New Biol.* **236:**52.

23. Yahara, I., and G. M. Edelman. 1972. Restriction of the mobility of lymphocyte immunoglobulin receptors by concanavalin A. *Proc. Natl. Acad. Sci. U.S.A.* **69:**608.

24. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology.* **14:**599.

25. Roelants, G., L. Forni, and B. Pernis. 1973. Blocking and redistribution ("capping") of antigen receptors on T and B lymphocytes by anti-immunoglobulin antibody. *J. Exp. Med.* **137:**1060.

26. Wilson, J. D., and J. F. A. P. Miller. 1971. T and B rosette forming cells. *Eur. J. Immunol.* **1:**501.

27. Rubin, B., and H. Wigzell. 1973. The immune response against hapten-autologous protein conjugates in the mouse. III. Specificity of cooperating nonthymus-processed (B) and thymus-processed (T) lymphocytes. *J. Exp. Med.* **137:**911.

28. Playfair, J. H. L., and S. Marshall-Clarke. 1973. Cross-reactions between erythrocytes at the T cell level. *Immunology.* **24:**579.

29. Cooper, M. G., and G. L. Ada. 1972. Delayed type hypersensitivity in the mouse. III. Inactivation of thymus derived effector cells. *Scand. J. Immunol.* **1:**247.

30. Feldman, M. 1972. Cell interactions in the immune response in vitro. V. Specific
collaboration via complexes of antigen- and thymus-derived cell immunoglobulin. *J. Exp. Med.* **136**:737.

31. Greaves, M. F., and N. M. Hogg. 1971. Immunoglobulin determinants on the surface of antigen-binding T- and B-lymphocytes in mice. *Prog. Immunol.* **1**:111.

32. Lee, S. T. and F. Paraskevas. 1972. Cell surface associated gamma globulins in lymphocytes. IV. Lack of detection of surface γ globulin on B-cells and acquisition of surface γG globulin by T-cells during primary response. *J. Immunol.* **109**:1262.

33. Orr, K. B., and F. Paraskevas. 1973. Cell surface associated gamma globulin in lymphocytes. V. Detection of early cytophilic complexes reacting with T- and B lymphocytes. *J. Immunol.* **110**:456.

34. Haskill, J. S., and M. A. Axelrad. 1971. Altered antigen-binding by immunocompetent cells as a reflection of immunological history. *Nat. New Biol.* **231**:219.

35. Marchalonis, J. J., R. E. Cone, and R. T. Rolley. 1973. Amplification of thymus influenced lymphocytes by poly-(A:U): inhibition of antigen binding by antisera to immunoglobulin light chain. *J. Immunol.* **110**:561.

36. Haskill, J. S. 1972. Discussion Paper: Studies on the differentiation within the immune system. *Ann. N. Y. Acad. Sci.* **207**:57.