FUNCTIONAL CHARACTERISTICS OF PEYER’S PATCH LYMPHOID CELLS*

I. INDUCTION OF HUMORAL ANTIBODY AND CELL-MEDIATED ALLOGRAFT REACTIONS

BY MARTIN F. KAGNOFF† AND STEPHEN CAMPBELL

(From the Department of Medicine, University of California at San Diego and The Salk Institute for Biological Studies, La Jolla, California 92037)

(Received for publication 15 October 1973)

Our aim is to determine the functional characteristics of Peyer’s patch lymphoid cells. Peyer’s patches are unique islands of lymphoid tissue in the mammalian small intestine which contain T cells and B cells (1–6). Although Peyer’s patch B cells can proliferate and mature into antibody-forming cells (3) there are conflicting reports concerning the immunocompetence of Peyer’s patch T cells (4, 7, 8). In situ, Peyer’s patch antigen-sensitive cells cannot be shown to produce an immune response regardless of the route of antigen administration (9, 10).

Induction of a primary humoral immune response to heterologous red blood cell antigens in mouse spleen cultures requires the presence of B cells as well as two accessory cell types (11, 12). B cells are the direct precursors of antibody-forming cells and possess membrane-associated immunoglobulin receptors which bind antigen (12). The first accessory cell type is a T cell, which in mice bears the alloantigen theta (13), while the second accessory cell type is found in an adherent cell population (14–16). It is recognized that accessory adherent cells are required also for induction in vitro of allograft reactions to cell surface alloantigens (17, 18).

To define Peyer’s patch lymphoid cells in functional terms, we examined the ability of Peyer’s patch cell suspensions to support the induction of humoral and cell-mediated immune responses. We report experiments which demonstrate that Peyer’s patch cultures from normal mice (C57BL/6, BALB/c) do not support the induction of primary humoral immune responses to heterologous red blood cells or the induction of cytotoxicity to allogeneic cells because they are deficient in an accessory adherent cell type(s). Humoral antibody synthesis and cell-mediated cytotoxic activity can be specifically

* This work was supported by National Institute of Arthritis, Metabolic and Digestive Disease Research Grant no. AM-70283-01, and National Institute of Allergy and Infectious Disease Research Grant no. AI-05875, and the Bernard L. Schwartz Fund.
† Dr. Kagnoff is recipient of a Clinical Investigator Award from the National Institute of Arthritis, Metabolic and Digestive Diseases.
induced in these cultures when adherent peritoneal exudate cells (APEC)\(^1\) or 2-mercaptoethanol (ME) are added. Peyer's patch cells from congenitally athymic (nude) mice are a highly purified B-cell population functionally devoid of T cells and accessory adherent cells. Our observations suggest that in normal mice, Peyer's patches contain antigen-sensitive B and T cells sequestered in such a way as to lack an accessory cell type or factor required for induction.

**Materials and Methods**

**Mice.**—BALB/c mice were obtained from the Salk Institute animal colony, San Diego, Calif. C57BL/6J, AKR/J, and C3HHeB/FeJ mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. Congenitally athymic (nude) mice considered deficient in T cells were obtained from Dr. J. Watson's colony, Salk Institute. The original breeding pairs for this colony were obtained from Dr. C. W. Friis, G. L. Blomholtgard Ltd., Ry, Denmark. This nude stock has been crossed onto a BALB/c background by mating heterozygous nude (nu/+)- males with inbred, homozygous BALB/c females (+/+). After three generations of backcrossing, heterozygous littermates were intercrossed. The resulting homozygous nude (nu/nu) female offspring were mated with inbred BALB/c males obtained from Jackson Laboratories and the inbreeding schedule was maintained by mating homozygous (nu/nu) females with inbred homozygous BALB/c males. In the experiments described in this paper, the homozygous (nu/nu) mice used were obtained in the following way. After one generation of backcrossing to inbred BALB/c males, heterozygous littermates were intercrossed (nu/+ X nu/nu). The heterozygous females and homozygous males in the offspring were used as parental mice (nu/+ X nu/nu) to produce homozygous nude (nu/nu) mice for these experiments, while heterozygous (nu/+ ) littermates which are phenotypically of the wild type were used as a source of T cells. All experiments used mice 7-9 wk old.

**Peyer's Patch Cultures.**—Peyer's patches from the small intestine were carefully dissected from the adjacent intestinal wall and teased to yield single cell suspensions.

**Induction of humoral immune responses:** Immune responses to red blood cell antigens were studied in Peyer's patch cell suspensions prepared from unimmunized nude C57BL/6 or BALB/c mice. The exact procedures described by Mishell and Dutton (19) for the study of mouse spleen cell suspensions were adapted for the study of Peyer's patch cell suspensions. Each Peyer's patch culture contained 1.0 X 10^7 cells in 1 ml Eagle's minimum essential medium (MEM) supplemented with 5% fetal bovine serum (FBS) and 3 X 10^6 sheep red blood cells (SRBC). Cultures of nude Peyer's patch cultures to which irradiated heterozygous nude Peyer's patch cells were added contained 5 X 10^6 nu/nu Peyer's patch cells. On the 4th day after culturing, the number of direct hemolytic plaque-forming cells (PFC) in each culture was determined using a microscope slide assay (19). The same SRBC used in culture were used in the slide assay. In addition, Peyer's patch cultures immunized with SRBC were examined for PFC directed against horse red blood cells (HRBC). Cell recoveries varied depending on whether nude or C57BL/6 cell suspensions were used. Therefore in all experiments we have expressed our data as the number of PFC per culture as averaged from duplicate cultures. Cell recoveries after 4 days ranged from 45 to 55% for C57BL/6 Peyer's patch cultures, and from 15 to 25% for nude Peyer's patch cultures. Cell viability was determined by trypan blue exclusion. No background PFC were observed in Peyer's patch cultures except when

---

1 *Abbreviations used in this paper:* APEC, adherent peritoneal exudate cells; BSS, balanced salt solution; FBS, fetal bovine serum; HRBC, horse red blood cells; ME, 2-mercaptoethanol; MEM, Eagle's minimum essential medium; FFC, plaque-forming cells; SRBC, sheep red blood cells; TSRBC, thymus cells activated to sheep red blood cells.
ME was added to cultures containing both B and T cells. Background PFC directed against SRBC in this case were less than 4% of the induced response and these background PFC have been subtracted from the reported data. Cultures were fed daily with a nutritional mixture supplemented with the FBS used in preparing the cultures (19).

Induction of allograft responses against H-2\(b\) antigens: The culture system used was identical to that described above, except that 2 \(\times\) 10\(^7\) BALB/c (H-2\(^b\)) Peyer's patch cells were cultured together with 3 \(\times\) 10\(^6\) Mitomycin C-treated C57BL/6 (H-2\(^b\)) spleen cells. C57BL/6 spleen cells were incubated with Mitomycin C (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 40 \(\mu\)g/ml for 30 min at 3\(^\circ\)C, and were washed twice with balanced salt solution (BSS) before use. Cultures were assayed for cytotoxic activity on the 5th day after culture. Cell recoveries range from 15 to 30%.

Cell-Mediated Cytotoxicity Assay.—The \(^{51}\)Cr release assay is a modification of that described by Brunner et al. (20).

Labeling of target cells: The C57BL/6 (H-2\(^b\))-derived ascites leukemia line EL4 and the DBA/2 (H-2\(^d\))-derived mastocytoma line P-815X-2 were maintained in continuous tissue culture and used as target cells in the cytotoxicity assay. Usually 5 \(\times\) 10\(^6\) to 10\(^7\) cells harvested in exponential growth were incubated with 100 \(\mu\)Ci \(^{51}\)Cr-labeled sodium chromate (The Radiochemical Center, Amersham, England) in a final volume of 1 ml Dulbecco's modified Eagle's medium supplemented with 10% FBS and glutamine for 30-40 min at 37\(^\circ\)C. The cells were washed twice, incubated for an additional 15 min, washed, and resuspended at 5 \(\times\) 10\(^4\) viable cells per ml.

Assay: On the 5th day after culture, cells from duplicate cultures were harvested, pooled, washed once, and resuspended at 10\(^5\) viable cells per ml in Dulbecco's modified MEM supplemented with 10% FBS and glutamine. For lymphocyte-target ratios of 100-1, 0.25 ml (2.5 \(\times\) 10\(^6\) cells) of the lymphocyte suspension was mixed with 50 \(\mu\)l (2.5 \(\times\) 10\(^4\) cells) of the \(^{51}\)Cr-labeled target cell suspension in a 12 \(\times\) 75 mm tube (Falcon 2052, Falcon Plastics, Div. of BioQuest, Los Angeles, Calif.), gassed with 10% CO\(_2\) in air, sealed with a silicone stopper, and rocked on a platform at 37\(^\circ\)C for 6 hr. In some instances, carrageenan (Marine Colloids, Inc., New York) suspended in BSS was added as indicated in the text. After 6 h 1 ml of cold BSS was added, the tubes were centrifuged, and the supernate and pellet were separated. Radioactivity of the supernates and pellets was determined in a Nuclear-Chicago gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Results are expressed as specific lysis which is corrected for background lysis. The background lysis of EL4, in the presence of normal unstimulated Peyer's patch cells was always in the range of 6-10% after 6 h of incubation while the background lysis of P-815 was in the range of 10-15%.

Fetal Bovine Serum.—FBS designated as normal (21) was taken from batch J76207, Reheis Chemical Corp., Chicago, Ill.

2-Mercaptoethanol.—ME was obtained from Matheson, Coleman and Bell, Norwood, Ohio, and used in culture at a concentration of 10\(^{-4}\) M.

Peritoneal Adherent Cells.—Unimmunized mice were injected intraperitoneally with 2.5 ml of sterile BSS. After 3 min the peritoneal fluid was aspirated, the cells were twice washed, counted, and resuspended at a concentration of 5 \(\times\) 10\(^8\) cells/ml in MEM containing 5% FBS. Cells in 1-ml aliquots were allowed to settle for 4 h at 37\(^\circ\)C in Falcon tissue culture dishes after which the supernatant fluid containing nonadherent cells was removed. Cells adhering to the tissue culture dish were twice rinsed with MEM containing 5% FBS. To these APEC 1 ml of a Peyer's patch cell suspension was added. Since it is impossible to determine the exact concentration of the remaining adherent cell fraction all the data of APEC concentrations refer to the number of originally seeded peritoneal exudate cells. C57BL/6, BALB/c, and nude APEC were used in cultures of C57BL/6, BALB/c, and nude Peyer's patches, respectively.

Irradiation of Peyer's Patch Cell Suspensions.—Peyer's patch cell suspensions from unimmunized C57BL/6 or heterozygous nude (nu/+) mice were irradiated in vitro with 2,000 R.
using a cobalt-60 source. These cell suspensions were used as a source of T cells as specified in the text (22, 23).

Activation of Thymus Cells.—C57BL/6 mice were irradiated (850 R) with a cobalt-60 source and then injected intraperitoneally with 0.1 ml heparin (Upjohn Co., Kalamazoo, Mich.). After 10 min these irradiated mice were injected intravenously with 0.2 ml of a solution containing $10^9$ thymus cells from nonirradiated C57BL/6 donors and $2 \times 10^8$ SRBC (24). After 6 days the spleen cells were isolated from the irradiated mice and used as the source of activated thymus cells. These cells are denoted TSRBC.

Adoptive Transfer.—C57BL/6 mice were irradiated (850 R) as above and then injected intraperitoneally with 0.1 ml heparin. After 10 min these irradiated mice were injected intravenously with 0.2 ml of a solution containing $6 \times 10^7$ Peyer's patch cells from nonirradiated unimmunized C57BL/6 donors and $2 \times 10^8$ SRBC. After 8 days the spleens were removed and the cell suspension assayed for PFC to SRBC as described above.

Antigens.—SRBC and HRBC were supplied by Colorado Serum Co., Denver, Colo.

Preparation of Antiserum.—Antiserum to theta antigen of C3H mice (antitheta C3H) was produced in AKR/J mice by repeated intravenous injections of C3HeB/FeJ thymus cells according to the procedure of Chan et al. (25). In the presence of guinea pig complement (Grand Island Biological Co., Grand Island, N. Y.) this AKR antitheta C3H serum diluted to 1:64 was more than 95% cytotoxic to thymus cells as determined by dye exclusion and at a dilution of 1:32 was found to be 30–35% cytotoxic to spleen cells and 40–60% cytotoxic to Peyer's patch cells. In vitro immune induction to SRBC could be restored in antitheta-treated cultures by the addition of TSRBC.

Antitheta Treatment.—$2 \times 10^7$ viable cells were suspended in 1 ml of a 1:5 dilution of antitheta serum, incubated at 0°C for 15 min, 37°C for 2 min, centrifuged, and the pellet incubated with 2 ml of a 1:5 dilution of guinea pig complement for 15 min at 37°C. Cells were then washed twice and used in the $^{51}$Cr release assay.

RESULTS

B- and T-Antigen-Sensitive Cells in C57BL/6 Peyer's Patches.—B- and T-cells were demonstrated in C57BL/6 Peyer's patches by adoptive transfer, or combined adoptive transfer and cell culture. (a) To demonstrate antigen-sensitive B cells, spleens removed from C57BL/6 mice which had been irradiated and injected with C57BL/6 donor Peyer's patch cells and SRBC 6 days previously were assayed for PFC directed against SRBC. These spleens contained greater than $10^6$ PFC directed against SRBC, while spleens from irradiated littermates injected with SRBC alone consistently contained no anti-SRBC PFC. (b) To demonstrate cooperating T-cell function, spleen cell suspensions were prepared from irradiated C57BL/6 mice which had been injected 6 days previously with syngeneic Peyer's patch cells and SRBC. These cell suspensions were irradiated in vitro with 2,000 R and added to cultures containing nude (nu/nu) spleen cells and SRBC. Nude spleen cultures to which primed Peyer's patch cells were added consistently yielded greater than 2,500 PFC directed against SRBC on day 4 of culture, while control nude spleen cultures to which SRBC alone were added yielded less than three anti-SRBC PFC.

In Vitro Induction of Immune Responses to SRBC in Cultures of Peyer's Patch Cells.—The data presented in Table I describe the requirements for
**Table I**

| Strain | Cell types in culture | Anti-SRBC PFC/culture |
|--------|-----------------------|-----------------------|
| nu/nu  | +                     | 0                     |
| nu/nu  | +                     | 1                     |
| nu/nu  | +                     | 0                     |
| C57BL/6| + + + +               | 2                     |
| nu/nu  | + + + + +             | 0                     |
| C57BL/6| + + + + +             | 0                     |
| C57BL/6| + + + + +             | 1739                  |
| nu/nu  | + + + + +             | 474                   |
| C57BL/6| + + + + + +           | 1,748                  |

* SRBC was used as the antigen in all cultures.
† nu/nu Peyer’s patches were considered a population of B cells while C57BL/6 Peyer’s patches were considered a population of B and T cells.
§ As a source of T cells for nu/nu cultures, 5 × 10^6 irradiated heterozygous nude (nu/+)-Peyer’s patch cells were added to 10^7 C57BL/6 Peyer’s patch cells as indicated.
|| 10^6, 5 × 10^6, 10^7, and 2 × 10^7 TSRBC were added to 10^7 C57BL/6 Peyer’s patch cells.

Induction of primary immune responses to SRBC in both C57BL/6 and nude Peyer’s patch cultures. The following was consistently observed: (a) PFC were not obtained in cultures of Peyer’s patch cells from nude mice. Addition of APEC or ME to these cultures did not result in the induction of anti-SRBC PFC. (b) PFC were not obtained in Peyer’s patch cultures from C57BL/6 mice previously shown to contain both T- and B-antigen-sensitive cells. Further, PFC were not obtained in Peyer’s patch cultures from nude mice to which irradiated heterozygous nude Peyer’s patch cells were added as a source of T cells. Finally, anti-SRBC PFC were not obtained in cultures of C57BL/6 Peyer’s patch cells to which 10^6, 5 × 10^6, 10^7, and 2 × 10^7 TSRBC were added. In contrast, PFC were induced when identical TSRBC were added to nude spleen cultures as controls. (c) Cultures of C57BL/6 Peyer’s patch cells to which either APEC or ME were added consistently yielded anti-SRBC PFC. Similarly, PFC were consistently induced in Peyer’s patch cultures containing both nude and irradiated heterozygous nude cells if either APEC (nu/nu) or ME were added. (d) Greater numbers of PFC directed against SRBC were obtained with the addition of ME than with the addition of APEC to culture.

**In Vitro Induction of Allograft Responses Against H-2b Antigens.**—The data presented in Table II describe the requirements for the in vitro induction of an allograft response against H-2^b antigens in Peyer’s patch cells. The following was observed: (a) Allograft responses could not be induced in Peyer’s patch cells when BALB/c (H-2^b) Peyer’s patch cells were cultured with several con-
TABLE II

In Vitro Induction of Allograft Responses in BALB/c Peyer's Patches

| Contents of culture | % specific lysis of target cells* |
|---------------------|----------------------------------|
| BALB/c (H-2d)       |                                  |
| Peyer's patch cells |                                  |
| C57BL/6 (H-2b)      |                                  |
| mitomycin C-treated |                                  |
| spleen cells        |                                  |
| BALB/c APEC         |                                  |
| ME                  |                                  |
| ELA (H-2b)          |                                  |
| P815X-2 (H-2d)      |                                  |

| BALB/c | C57BL/6 (H-2b) | 10^6 | 10^6 | 10^6 | 10^6 | 10^6 | 10^6 | 10^6 | 10^6 |
|--------|----------------|------|------|------|------|------|------|------|------|
|        | mitomycin      |      |      |      |      |      |      |      |      |
|        | C-treated      |      |      |      |      |      |      |      |      |
|        | spleen cells   |      |      |      |      |      |      |      |      |
|        | BALB/c APEC    |      |      |      |      |      |      |      |      |
|        | ME             |      |      |      |      |      |      |      |      |
|        | ELA            |      |      |      |      |      |      |      |      |
|        | P815X-2        |      |      |      |      |      |      |      |      |

* Expressed as mean of triplicate assay tubes ±SE.

centations of C57BL/6 (H-2b) spleen cells. (b) Specific allograft responses could be induced in BALB/c Peyer's patches when 10^-4 M ME or APEC were added to culture. (c) Allograft responses with the addition of ME were consistently greater than with the addition of APEC.

The cell type induced in culture to mediate cytotoxic activity appeared to be a thymus-derived cell since: (a) Treatment of sensitized Peyer's patch cells with antitheta serum and complement decreased cytotoxicity for the target cells by 96%. No decrease in cytotoxicity was found in control cultures treated with complement alone. (b) Addition of 50 μg/ml carrageenan to the assay did not reduce the cytotoxicity of sensitized Peyer's patch cells for the EL4 target cells. This concentration of carrageenan did inhibit antibody-complement mediated lysis of SRBC in vitro. (c) Supernates from sensitized Peyer's patch cultures did not exhibit lytic capacity against target cells with or without the addition of complement.

DISCUSSION

Our data permit several new observations: (a) Peyer's patch lymphoid cells from normal mice (C57BL/6 or BALB/c) are functionally deficient in the accessory adherent cell type required for the induction of a humoral immune response to SRBC (14–16), and for the induction of an allograft response against cell surface alloantigens (17, 18). Specific humoral and cell-mediated immune responses can be induced in these Peyer's patch cultures only when APEC or ME are added. (b) Peyer's patches from normal mice contain antigen-sensitive T cells which can cooperate in B-cell induction. This is shown in three experiments. First, primary immune responses can be induced by SRBC in nude spleen cultures after the addition of irradiated allogeneic Peyer's patch T cells activated to SRBC. In this case, T-cell cooperation may occur via the normal inductive pathway or via an allogeneic effect (26, 27). Second, irradiated heterozygous nude (nu/+) Peyer's patch cells which are considered a
source of T cells restore the ability to induce a SRBC response in nude (nu/nu) Peyer's patch cultures containing APEC or ME. Third, the induction of an anti-SRBC response in C57BL/6 Peyer's patch cultures containing APEC or ME is prevented when T cells are removed by antitheta treatment. (c) Peyer's patch cells from normal mice contain antigen-sensitive T cells which can be induced to mediate allograft responses against cell surface alloantigens (i.e., killer function). (d) Peyer's patches from normal and nude mice contain functional antigen-sensitive B cells. Like spleen cultures, Peyer's patch cultures require the presence of B cells, cooperating T cells, and an accessory adherent cell type or ME for the induction of primary immune responses to SRBC. Since nude Peyer's patches functionally contain only antigen-sensitive B cells, they may be a useful tool for future studies requiring a functionally purified B cell population.

One further observation should be pointed out. ME substitutes for APEC in restoring both humoral and cell-mediated immune responsiveness in Peyer's patch cultures deficient in an adherent cell type(s). This ME effect requires the presence of T cells. Previously, ME was shown to substitute for adherent cells in the induction of humoral antibody responses in spleen cell cultures (28). ME and other thiol compounds can affect cell growth (29). Further, cell viability in spleen cell cultures lacking adherent cells is significantly lower than in normal spleen cell cultures or cultures deficient in adherent cells to which ME is added (28). In contrast, viability of Peyer's patch cells in our experiments is increased only slightly by ME and is not increased by the addition of APEC to culture.

Peyer's patches cannot be induced to produce immune responses regardless of the route of immunization in vivo (9, 10). The apparent paradox that Peyer's patches contain reactive T and B cells, yet do not respond to antigen in vivo, places this lymphoid cell population in a unique position among the peripheral lymphoid tissue. We suggest that in normal mice Peyer's patch aggregates are a storehouse of antigen-sensitive T and B cells sequestered in such a way as to lack an accessory cell type or factor required for induction.

SUMMARY

Peyer's patches from normal mice contain antigen-sensitive B and T cells, but lack the accessory adherent cell type(s) required both for the induction of humoral immune responses and for the induction of allograft reactions against cell surface alloantigens. Immune responsiveness can be restored to cultures of Peyer's patch cells by the addition of either APEC or ME. Peyer's patch B cells can be specifically induced by antigen to synthesize humoral antibody. Peyer's patch T cells can cooperate in B-cell induction and can be induced to mediate an allograft reaction against an allogeneic stimulus. Peyer's patch lymphoid aggregates appear to be a storehouse of antigen-sensitive cells sequestered in such a way as to lack an accessory cell type or factor required for induction.
We are grateful to Dr. Melvin Cohn for his generous support. We also thank Dr. Melvin Cohn and Dr. James Watson for their comments on this manuscript, and Ms. B. Claudy for expert technical assistance.

REFERENCES

1. Raft, M. C., and J. J. T. Owen. 1971. Thymus-derived lymphocytes: their distribution and role in the development of peripheral lymphoid tissues of the mouse. Eur. J. Immunol. 1:27.

2. Chanana, A. D., J. Schaedeli, M. W. Hess, and H. Cottier. 1973. Predominance of theta-positive lymphocytes in gut-associated and peripheral lymphoid tissues of newborn mice. J. Immunol. 110:283.

3. Craig, S. W., and J. J. Cebra. 1971. Peyer's patches: an enriched source of precursors for IGA-producing immunocytes in the rabbit. J. Exp. Med. 134:188.

4. Levin, D. M., D. L. Rosenstreich, and H. Y. Reynolds. 1973. Immunologic responses in the gastrointestinal tract of the guinea pig. I. Characterization of Peyer's patch cells. J. Immunol. 111:980.

5. Joel, D. D., M. W. Hess, and H. Cottier. 1972. Magnitude and pattern of thymic lymphocyte migration in neonatal mice. J. Exp. Med. 135:907.

6. Waksman, B. H. 1973. The homing pattern of thymus-derived lymphocytes in calf and neonatal mouse Peyer's patches. J. Immunol. 111:878.

7. Heim, L. R., M. P. McGarry, J. R. Montgomery, J. J. Trentin, and M. South. 1972. Potentials of spleen, lymph node, and Peyer's patches to reconstitute lymphoid tissue and produce graft-versus-host reaction. Transplantation. 14:418.

8. Perey, D. Y. E. and R. D. Guttmann. 1972. Peyer's patch cells. Absence of graft-versus-host reactivity in mice and rats. Lab. Invest. 27:427.

9. Bienenstock, J., and J. Dolezel. 1971. Peyer's patches: lack of specific antibody-containing cells after oral and parenteral immunization. J. Immunol. 106:938.

10. Henry, C., W. P. Faulk, L. Kuhn, J. M. Yoffey, and H. H. Fudenberg. 1970. Peyer's Patches: immunologic studies. J. Exp. Med. 131:1200.

11. Claman, H. N., E. A. Chaperon, and R. F. Triplett. 1966. Immunocompetence of transferred thymus-marrow cell combinations. J. Immunol. 97:828.

12. Miller, J. F. A. P., and G. F. Mitchell. 1969. Thymus and antigen-reactive cells. Transplant. Rev. 1:3.

13. Raff, M. C. 1970. Role of thymus-derived lymphocytes in the secondary humoral immune response in mice. Nature (Lond.) 226:1257.

14. Mosier, D. E., and L. W. Coppleston. 1968. A three-cell interaction required for the induction of the primary immune response in vitro. Proc. Natl. Acad. Sci. U. S. A. 61:542.

15. Cosenza, H., L. D. Leserman, and D. A. Rowley. 1971. The third cell type required for the immune response of spleen cells in vitro. J. Immunol. 107:414.

16. Feldmann, M. 1972. Cell interactions in the immune response in vitro. II. The requirement for macrophages in lymphoid cell collaboration. J. Exp. Med. 136:1049.

17. Wagner, H., M. Feldmann, W. Boyle, and J. W. Schrader. 1972. Cell-mediated immune response in vitro. III. The requirement for macrophages in cytotoxic reactions against cell-bound and subcellular alloantigens. J. Exp. Med. 136:331.

18. MacDonald, H. R., R. A. Phillips, and R. G. Miller. 1973. Allograft immunity
in the mouse. II. Physical studies of the development of cytotoxic effector cells from their immediate progenitors. *J. Immunol.* **111**:575.

19. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* **126**:423.

20. Brunner, K. T., J. Mauel, H. Rudolf, and B. Chapuis. 1970. Studies of allograft immunity in mice. I. Induction, development and in vitro assay of cellular immunity. *Immunology*. **18**:501.

21. Watson, J., and R. Epstein. 1973. The role of humoral factors in the initiation of in vitro primary immune responses. I. Effects of deficient fetal bovine serum. *J. Immunol.* **110**:31.

22. Kettman, J., and R. W. Dutton. 1971. Radioresistance of the enhancing effect of cells from carrier-immunized mice in an in vitro primary immune response. *Proc. Natl. Acad. Sci. U. S. A.* **68**:699.

23. Hamaoka, T., D. H. Katz, and B. Benacerraf. 1972. Radioresistance of carrier-specific helper thymus-derived lymphocytes in mice. *Proc. Natl. Acad. Sci. U. S. A.* **69**:3483.

24. Vann, D. C., and J. R. Kettman. 1972. In vitro cooperation of cells of bone marrow and thymus origin in the generation of antibody-forming cells. *J. Immunol.* **108**:73.

25. Chan, E. L., R. I. Mishell, and G. F. Mitchell. 1970. Cell interaction in an immune response in vitro: requirement for theta-carrying cells. *Science (Wash. D. C.).* **170**:1215.

26. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1971. Carrier function in antihapten antibody responses. III. Stimulation of antibody synthesis and facilitation of hapten-specific secondary antibody responses by graft-versus-host reactions. *J. Exp. Med.* **133**:169.

27. Lefkovits, I. 1973. In vitro complementation experiments with nude mice. I. The allogeneic effect in the antibody response to sheep red cells. *Eur. J. Immunol.* **3**:397.

28. Chen, C., and J. G. Hirsch. 1972. Restoration of antibody-forming capacity in cultures of nonadherent spleen cells by mercaptoethanol. *Science* **176**:560.

29. Broome, J. D., and M. W. Jeng. 1973. Promotion of replication in lymphoid cells by specific thiols and disulfides in vitro. *J. Exp. Med.* **138**:574.