POTENTIATION OF THE T-LYMPHOCYTE RESPONSE TO MITOGENS

II. THE CELLULAR SOURCE OF POTENTIATING MEDIATOR(S)*

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The first papers of the present series establish that mouse thymocytes and peripheral thymus-processed (T) lymphocytes are stimulated to mitosis and their response to such agents as phytohemagglutinin greatly potentiated by factor(s) designated “lymphocyte-activating factor” (LAF), which are produced in cultures of syngeneic or xenogeneic lymphoid cells (1, 2). In this paper, we present data showing that adherent cells, probably macrophages, are the principal source of LAF and that its production is increased by agents which stimulate these cells.

Materials and Methods

Animals.—Male or female CBA/J mice, 6–12 wk of age, were used without treatment or after irradiation (850 R) and reconstitution with $5 \times 10^5$ syngeneic bone marrow cells (XBMs), $5-8 \times 10^3$ thymocytes (XT), or both bone marrow and thymus cells (XBMTs). Young adult New Zealand albino rabbits of both sexes were purchased from a local dealer and used without treatment.

Cell Preparation and Culture.—All the materials and techniques employed for cell preparation and culture are fully described in our previous papers (1–3). The separation of adherent from nonadherent cells was carried out both on plastic (4, 5) and by the use of nylon columns (6, 7). While 8% pooled normal human serum was routinely used for lymphocyte culture, the adherence technique required higher concentrations, 10% human serum being used routinely and 10% fetal calf serum (Grand Island Biological Company, Grand Island, N.Y.) in one experiment.

RESULTS

Release of Potentiating Factors by Cells from Different Sources.—Factors stimulatory to normal CBA thymocytes are released by normal human, rabbit,

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Abbreviations used in this paper: B, nonthymus processed; Con A, concanavalin A; LAF, lymphocyte-activating factor; LPS, lipopolysaccharide; PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin; SUP, supernatant; T, thymus processed; T-3H, thymidine-3H; XBM, irradiation plus syngeneic bone marrow cells; XBMT, irradiation plus syngeneic bone marrow cells and thymocytes; XT, irradiation plus syngeneic thymocytes.
or syngeneic mouse lymphoid cells into the supernatant (SUP) (Table I). Human peripheral blood lymphocytes (PBL), acted on by phytohemagglutinin (PHA) or lipopolysaccharide (LPS), produce SUP which is mitogenic alone and which greatly potentiates the thymocyte response to PHA. The production of SUP activity bears no relation to DNA synthesis in the donor culture, which is marked with PHA and negligible with respect to LPS. Concanavalin A (Con A), which was mitogenic for the donor culture, gave no SUP activity in this experiment.

With syngeneic spleen cells, all three mitogens stimulate mitosis and production of SUP activity but at a lesser level than with human cells. Only Con

| Experiment | Donor culture | Recipient culture | T-3H uptake | SUP alone | SUP + PHA |
|------------|---------------|------------------|-------------|-----------|-----------|
| I None     | Human blood leukocytes | None | 63 | 28 | 488 |
|            | PHA           | 64,492 | 14,880 | 38,418 |
|            | LPS           | 747 | 13,426 | 53,898 |
|            | Con A         | 2,426 | 73 | 368 |
|            | Mouse spleen cells | None | 355 | 42 | 417 |
|            | PHA           | 17,003 | 109 | 1,224 |
|            | LPS           | 55,697 | 147 | 14,811 |
|            | Con A         | 85,200 | 1,091 | 9,090 |
| II None    | Rabbit spleen cells | None | 1,391 | 265 | 1,305 |
|            | PHA           | 6,154 | 519 | 1,551 |
|            | LPS           | 1,856 | 520 | 4,637 |

* Mouse thymocytes.

A-SUP stimulates DNA synthesis in recipient thymocytes without added PHA. Rabbit spleen cells give similar findings but a still lower level of SUP production. However, rabbit cells were agglutinated to some extent by human serum in the medium, and SUP production may have been affected by this. LPS was clearly the best stimulant of SUP production with all the cell types tested.

When mouse organs are compared (Table II), bone marrow, spleen, and thymus, in that order, are found to contain cells active in producing SUP. Some is released by unstimulated cells, more by cells exposed to PHA or Con A, and the most by cells treated with LPS. Only LPS was effective in stimulating marrow to produce an active SUP. While all the supernatants tested acted synergistically with PHA, only Con A-SUP of spleen and LPS-SUP from
marrow were mitogenic alone, in each case more so than PHA. Again there was no correlation between donor cell mitosis and production of SUP.

**Characterization of Cells which Produce Potentiating Factors.**—Lethal irradiation of mouse donors and reconstitution with bone marrow alone did not significantly affect the ability of their spleen cells to produce SUP, when incubated without stimulant or with LPS (Table III). With PHA and Con A, on the other hand, there was some loss of ability to form SUP, and this was restored by the additional injection of thymocytes. These data and those of

| Experiment | Donor culture | Recipient culture |
|------------|---------------|------------------|
| Cell source | Stimulant | T-\(^{3}H\) uptake | SUP alone | SUP + PHA |
| Spleen     | None          | 1,763            | 80        | 1,367 |
|            | PHA           | 42,061           | 183       | 3,919 |
|            | LPS           | 60,635           | 419       | 13,568 |
|            | Con A         | 112,606          | 5040      | 12,984 |
| Thymus     | None          | 35               | 76        | 803   |
|            | PHA           | 456              | 146       | 1,141 |
|            | LPS           | 337              | 247       | 3,892 |
|            | Con A         | 7,108            | 199       | 886   |
| Spleen     | None          | 231              | 56        | 3,046 |
|            | PHA           | 35,780           | 364       | 4,285 |
|            | LPS           | 61,310           | 632       | 23,529 |
|            | Con A         | 158,447          | 7125      | 25,406 |
| Bone marrow| None          | 3,336            | 45        | 2,741 |
|            | PHA           | 11,143           | 209       | 4,073 |
|            | LPS           | 10,663           | 5008      | 33,676 |
|            | Con A         | 11,328           | 369       | 4,883 |

Table II imply that T lymphocytes may contribute to the formation of active SUP with PHA and Con A but not with LPS.

When mouse spleen cells were separated into adherent and nonadherent populations by incubation in plastic Petri dishes, the ability to produce mitogenic and potentiating SUP, after stimulation with Con A and especially with LPS, was found to reside almost entirely in the adherent cells (Table IV). When the adherent cells were cultured for 2 days and washed repeatedly to remove cells other than macrophages, they remained active as sources of SUP. At this time contamination with granulocytes and lymphocytes is minimal.

**Human PBL.** were similarly freed of adherent cells by passage through nylon
columns. These cells retained their reactivity with PHA, as shown by thymi-
dine-\(^{3}H\) (T-\(^{3}H\)) uptake after 3 days in culture, yet lost to a considerable degree
their ability to produce mitogenic or potentiating factors when stimulated
with either PHA or LPS (Table V). Titrations indicated that column-purified

**TABLE III**

Response to Stimulants and Production of Potentiating Factors by Syngeneic Normal, XBM,
and XBMT Spleen Cells

| Treatment of mouse | Donor culture | Recipient culture T-\(^{3}H\) uptake |
|-------------------|--------------|----------------------------------|
|                   |              | SUP alone | SUP + PHA |
| Normal            | None         | 221       | 45 879 |
|                   | PHA          | 22,025    | 23 1,015 |
|                   | LPS          | 38,201    | 8,432 |
|                   | Con A        | 115,712   | 14,364 |
| XBM               | None         | 1,151     | 44 1,008 |
|                   | PHA          | 6,484     | 95 2,458 |
|                   | LPS          | 1,382     | 179 11,829 |
|                   | Con A        | 10,653    | 641 5,137 |
| XBMT              | None         | 2,552     | 38 2,746 |
|                   | PHA          | 21,705    | 233 6,227 |
|                   | LPS          | 4,872     | 1048 13,832 |
|                   | Con A        | 50,981    | 3028 13,702 |

**TABLE IV**

Production of Potentiating Mediators by Adherent and Nonadherent Mouse Spleen Cells

| Stimulant of donor culture | PHA in recipient culture | Mouse spleen fraction |
|---------------------------|--------------------------|-----------------------|
|                           |                          | Original | Nonadherent | Adherent (1 hr) | Adherent (2 days) |
| None                      | -                        | 30*      | 48          | 37          | ND             |
|                           | +                        | 238      | 157         | 789         | ND             |
| LPS                       | -                        | 76       | 42          | 3,112       | 173            |
|                           | +                        | 2258     | 759         | 15,326      | 7992           |
| Con A                     | -                        | 401      | 92          | 541         | ND             |
|                           | +                        | 1662     | 258         | 3,665       | ND             |

* Values for T-\(^{3}H\) uptake (counts per minute) in recipient thymocyte cultures without
added SUP were unstimulated, 55, and PHA-stimulated, 293.

cells produced less than 4% of the SUP activity released by the original un-
fractionated PBL. The residual activity did not disappear on dilution and may
differ qualitatively from that obtained with unpurified cells.

The Indirect Action of Mitogens.—Removal of adherent cells from a
normal CBA thymocyte population reduces the response of these cells in
culture (T-\(^{3}H\) uptake) to PHA and Con A to less than one-half of control values
and virtually eliminates the response to LPS (Table VI). The supernatants after treatment of control cultures with any of the three mitogens show SUP activity, and comparable activity is much reduced in the supernatants of nonadherent cells in culture (data not shown).

Quantitative Comparison of Different Supernatants.—A comparative titration was carried out on two active SUP obtained from human PBL stimulated with PHA and LPS (Fig. 1). Both were mitogenic alone for mouse thymocytes, and both enhanced the response of these cells to PHA. Enhancement was signifi-

TABLE V
Effect of Nylon Column Purification on Ability of Human Blood Leukocytes to Produce Potentiating Factors

| Experiment | Donor culture | SUP dilution | Recipient culture | T-11 uptake |
|------------|--------------|--------------|------------------|-------------|
|            | Cells Stimulant | SUP alone | SUP + PHA |
| I None     | — — —           | 64 1,032  | |
| Original   | None 29 Undiluted | 3,748 | 30,139 |
|            | 1:5             | 202 12,853 | |
|            | LPS Undiluted  | 19,942 | 51,858 |
|            | 1:5             | 1,214 20,073 | |
|            | 1:25            | 155 6,148 | |
| Purified   | None 38 —       | ND ND | |
|            | PHA 7,899 Undiluted | 125 | 1,900 |
|            | 1:5             | 57 1,718 | |
|            | LPS 21 Undiluted | 122 | 3,655 |
|            | 1:5             | 100 3,084 | |
| II None    | — — —           | 53 525 | |
| Original   | None 60 Undiluted | 87 | 833 |
|            | PHA 45,855 Undiluted | 27,529 | 31,518 |
| Purified   | None —          | ND ND | |
|            | PHA 26,210 Undiluted | 200 | 1,409 |

cant at concentrations of SUP below the mitogenic threshold. The maximal level of enhanced stimulation attained was only slightly greater in each case than that achieved with the optimal concentration of SUP alone. The titration curves, both for SUP alone and for SUP + PHA, were closely similar and suggested that the same active agent was present in the two preparations.

DISCUSSION

In the previous paper (2), evidence was presented that the target cell for LAF is the central or peripheral thymus-processed (T) lymphocyte. The data of the present paper show that LAF is probably produced by macrophages in
both human and mouse cultures. Active SUP were obtained from adherent cells, which could be maintained for 2 days attached to plastic and which withstood vigorous washing. Such preparations do not include significant numbers of T cells, nor do spleen cell suspensions from XBM mice, which also

TABLE VI

| Stimulant       | T-TH uptake by thymus cells | Ratio original/nonadherent |
|-----------------|-----------------------------|----------------------------|
| None            | 130                         | 20                         | 6.5 |
| LPS             | 343                         | 81                         | 4.2 |
| PHA             | 441                         | 178                        | 2.5 |
| LPS + PHA       | 1527                        | 274                        | 5.6 |
| Con A           | 5844                        | 2606                       | 2.2 |

* Collected after two incubations in Petri dishes.

![Fig. 1. Titration of mitogenic and potentiating activity of SUP prepared by incubating PBL with PHA (▲) or LPS (△). T-TH-incorporation of mouse thymocytes without additives ..., with PHA ---, with SUP --, or with SUP + PHA ---.](image)

produced SUP as active as that from normal spleen. T cells may play an indirect role, nevertheless, when PHA or Con A is used to stimulate LAF production by macrophages (see below), and this may account in part for differences in LAF production by different organs stimulated with the various agents. Nonthymus processed (B) cells appear to be ruled out as a source of LAF since active SUP could be obtained from thymus cell suspensions and from
XBM spleen at a time when no B cells reactive to LPS are present (3). The most active SUP were obtained with LPS, a well-recognized stimulant of macrophages (8-11).

There is no evidence that LAF produced by "unstimulated" adherent cells differs qualitatively from that produced after stimulation with PHA or LPS or that the latter are different from each other. All are mitogenic alone and show striking synergy with PHA or with Con A used at concentrations below those giving a maximal response. Their titration curves, alone or in the presence of PHA, are perfectly parallel (Fig. 1). Apparent qualitative differences in SUP from different animal species and different organs are most readily explained as depending on differences in LAF concentration. Also, as noted, differences in the number of T cells influence LAF production by PHA or Con A as compared with LPS. There may nevertheless be some heterogeneity of factors which act on lymphocytes. We have seen (Table V) that nylon column-purified PBL can be stimulated by LPS to produce weakly active SUP whose activity resists dilution. Tridente et al. (12, 13) and Winkelstein (14) have described synergistic responses of thymocytes with other cells having different kinetic properties from those under consideration here, and the former group reports that the adherent cells from bone marrow are less effective in stimulating thymocytes than unfractionated marrow cells.

The present observations suggest that LAF may play an essential role in many or all the immunologic responses in which T cells participate, certainly in each instance where a requirement for macrophages or "adherent cells" has been demonstrated. Such a requirement in the reaction of the sensitized cells of delayed hypersensitivity with eliciting antigen is well recognized, whether this be measured by proliferation and blast transformation (6) or by production of secondary mediators such as lymphotoxin. Equally important is the primary response of unimmunized T cells, as in the graft-versus-host reaction and its in vitro equivalent, the mixed lymphocyte reaction (15, 16), or the response to an immunizing dose of soluble antigen with adjuvant (17). In such primary responses, a further cooperation between T cells of different biologic types or specificities may be essential (18, 19). Finally, the cooperation of T and B cells (20, 21) in stimulating the latter to plasma cell transformation and antibody production requires macrophages both in vivo (22) and in vitro (4, 5, 23-27). In several of these instances involving T cells alone (16, 28) or T-B cell cooperation (24, 25, 29) it has already been recognized that soluble factors comparable to LAF serve as mediator. This conclusion does not necessarily conflict with other suggested mechanisms of macrophage action in the immune response. Supernatant factors described by other investigators appear in some instances to represent altered antigen (25, 29). There is per-
suasive evidence that antigen attached in or near the cell surface of macrophages may act as "superantigen" (30-33) or that antigen linked to a special RNA provided by the macrophage may play this role (34-38).

One of the most important implications of the present observations concerns the role of macrophages in the action of adjuvants, whether to increase delayed sensitization or to enhance antibody production. In the intact animal (10, 39) some adjuvant materials, such as mineral oil, simply promote wide dissemination and long persistence of the antigenic stimulus. Others increase the flux of lymphocytes into thymus-dependent areas of stimulated peripheral lymph nodes (40), a localization which may depend on specific as well as nonspecific elements in the local response to antigen and adjuvant and a release of mediators (41, 42). Adjuvant effects are also observed in vitro however (43), and clearly must be mediated by more direct cellular events. Enhanced antigen uptake by macrophages (44-46), whether as a result of the particulate character of the antigen (47, 48) or of stimulation directed to the macrophage (49, 50), was long felt to account for such effects. This mechanism accords well with the suggested role of the macrophage in providing superantigens to the host (30, 31, 33-37). More recently, however, the suggestion was made that adjuvants act by labilizing macrophage lysosomes (51-53), and an increased production of lysosomal enzymes does in fact result from the action of LPS on macrophage monolayers (11). A target for the released lysosomal contents has not been identified; it is possible that LAF may be a lysosomal factor the target of which is the T lymphocyte. T cells in XT mice synthesize DNA rapidly in response to in vivo stimulation with a variety of adjuvant materials, among them pertussis vaccine and purified LPS. Our findings, presented in this and the preceding paper, establish that the stimulus to these cells must be mediated in part by macrophages and LAF production. These findings assume greater significance in the light of the recent demonstration that the adjuvant effect of pertussis, LPS, Freund's adjuvant, or retinol on antibody formation requires the participation of T cells (17, 42) and the in vitro demonstration that phagocytic cells and T lymphocytes together are required to "help" nonmitotic B cells respond to antigens such as sheep erythrocytes (24, 29, 54).

Certain stimulatory effects observed in the intact animal and in vitro appear to be indirect. PHA and Con A produce less effective SUP from bone marrow than spleen, and its production in the latter is lessened in XBM animals as contrasted with XBMT animals (see Tables II and III). This implies that these mitogens, unlike LPS which is highly effective in producing LAF activity from bone marrow and in the presumed absence of T cells, act in part by stimulating T lymphocytes which then activate macrophages with their mediators and these in turn produce LAF, a striking circular mechanism. PHA and Con A act only on T cells (55-59). That stimulation of T cells may produce activation of macrophages indirectly by way of soluble mediators has been
demonstrated repeatedly (60–62). Thus the postulated circular mechanism may come into play in any situation where T cells are activated, for example in the interesting “allogeneic effect” described by Katz et al. (63). The fact that mouse spleen reacts maximally to a dose of Con A 5–10-fold lower than required for maximal stimulation of mouse thymus may simply reflect the relative paucity of macrophages in the thymus and consequent weakness of the suggested circular mechanism. Removal of macrophages and other large cells from reactive thymocytes on bovine serum albumin gradients has been shown (2, 64) to diminish their reactivity to PHA. PHA and Con A, however, may also have direct effects on macrophages, and we have shown production of LAF from adherent cells by the former (59). In consequence, as one would predict, PHA acts as an adjuvant if administered at the correct time in relation to antigen (see references 65 and 66 for reviews).

The response to LPS also illustrates a second type of indirect effect. It is well recognized (8–10) that LPS is highly stimulatory to macrophages both in vivo (10) or in vitro (11). In the mouse, LPS also stimulates B lymphocytes but has no direct action on T cells (3, 59, 67). In accord with these findings, the stimulation of thymocytes by LPS is shown in the present paper to depend entirely on the presence of adherent cells, presumably macrophages. One must suppose that the vigorous proliferative response of peripheral T cells when LPS is given to XT animals may also be dependent on production of small amounts of LAF which stimulate these cells.

**SUMMARY**

Effective supernatants (SUP), which potentiate mouse T-cell responses to phytohemagglutinin (PHA), are obtained from cells of several species (human, rabbit, rat, mouse) and indeed from syngeneic spleen, thymus, or bone marrow cells. Unstimulated cells release some SUP activity but more is produced after stimulation. Lipopolysaccharide (LPS) produced very active SUP in all cultures tested. PHA was similarly active on human leukocytes only, whereas concanavalin A (Con A) gave highly efficient SUP only with mouse spleen cells. SUP production is not correlated with a mitotic response of the donor cells and is observed in cultures unable to respond mitotically to the stimulant. Adherent mouse spleen cell populations, consisting largely or entirely of macrophages, produce active SUP, while nonadherent cells do not. Similarly, purification of human peripheral leukocytes on nylon columns, with removal of macrophages and other adherent cells, destroys their ability to produce SUP. The importance of indirect effects in stimulating mitotic responses of T cells is emphasized by the fact that the mitotic response of mouse thymocytes to LPS and its ability to potentiate the response of these cells to PHA disappears with removal of adherent cells from the thymocyte population. Con-

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*Gery, I. Unpublished data.*
versely the production of SUP from spleen cells stimulated by Con A requires the presence of T cells.

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BIBLIOGRAPHY

1. Gery, I., R. K. Gershon, and B. H. Waksman. 1971. Potentiation of cultured mouse thymocyte responses by factors released by peripheral leukocytes. J. Immunol. 107:1778.
2. Gery, I., R. K. Gershon, and B. H. Waksman. 1972. Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. J. Exp. Med. 138:128.
3. Gery, I., J. Kruger, and S. Z. Spiesel. 1972. Stimulation of B-lymphocytes by endotoxin. Reactions of thymus-deprived mice and karyotypic analysis of dividing cells in mice bearing T6T6 thymus grafts. J. Immunol. 108: 1088.
4. Mosier, D. E. 1967. A requirement for two cell types for antibody formation in vitro. Science (Wash. D.C.). 158:1573.
5. Tan, T., and J. Gordon. 1971. Participation of three cell types in the anti-sheep red blood cell response in vitro. Separation of antigen-reactive cells from the precursors of antibody-forming cells. J. Exp. Med. 133:520.
6. Oppenheim, J. J., B. G. Leventhal, and E. M. Hersh. 1968. The transformation of column-purified lymphocytes with nonspecific and specific antigenic stimuli. J. Immunol. 101:262.
7. Amos, D. B. 1969. Cytotoxicity testing. In Manual of Tissue Typing Techniques. Compiled by Transplantation Immunology Branch Collaborative Research, NIAID, NIH. 1.
8. Bennett, I. L., Jr., and L. E. Cluff. 1957. Bacterial pyrogens. Pharmacol. Rev. 9:427.
9. Nowotny, A. 1969. Molecular aspects of endotoxic reactions. Bacteriol. Rev. 33:72.
10. Neter, E. 1969. Endotoxins and the immune response. Curr. Top Microbiol. Immunol. 47:82.
11. Wiener, E., and D. Levanon. 1968. The in vitro interaction between bacterial lipopolysaccharide and differentiating monocytes. Lab. Invest. 19:584.
12. Trindade, G., G. Biasi, L. Chieco-Bianchi, and L. Fiore-Donati. 1971. Thymus-marrow cell interaction in vitro. Enhancing effect of different bone marrow constituents on PHA response of thymocytes. In Morphological and Functional Aspects of Immunity. K. Lindahl-Kiessling, G. Alm, and M. G. Hanna, Jr., editors. Plenum Publishing Corporation, New York. 633.
13. Trindade, G., G. Biasi, L. Chieco-Bianchi, and L. Fiore-Donati. 1971. Thymus-marrow cell interaction evaluated by PHA stimulation and graft-versus-host activity. Nature (Lond.). 234:105.
14. Winkelstein, A. 1971. Augmentation of PHA responsiveness in mixed thymus-marrow cultures. J. Immunol. 107:195.
15. Alter, B. J., and F. H. Bach. 1970. Lymphocyte reactivity in vitro. I. Cellular reconstitution of purified lymphocyte response. Cell. Immunol. 1:207.
16. Bach, F. H., B. J. Alter, S. Solisay, D. C. Zoschke, and M. Janis. 1970. Lymphocyte reactivity in vitro. II. Soluble reconstituting factor permitting response of purified lymphocytes. Cell. Immunol. 1:219.
17. Allison, A. C., and A. J. S. Davies. 1971. Requirement of thymus-dependent lymphocytes for potentiation by adjuvants of antibody formation. *Nature (Lond.)* **233**:330.

18. Cantor, H., R. Asofsky, and N. Talal. 1970. Synergy among lymphoid cells mediating the graft-versus-host response. II. Synergy in graft-versus-host reactions produced by BALB/c lymphoid cells of differing anatomic origin. *J. Exp. Med.* **131**:235.

19. Raff, M. C., and H. I. Cantor. 1971. Subpopulations of thymus cells and thymus-derived lymphocytes. In Progress in Immunology. D. B. Amos, editor. Academic Press, Inc., New York. 83.

20. Möller, G., editor. 1969. Antibody-sensitive cells. *Transplant. Rev.* **1**:3.

21. Playfair, J. H. L. 1971. Cell cooperation in the immune response. *Clin. Exp. Immunol.* **8**:839.

22. Talmage, D. W., J. Radovich, and H. Hemmingsen. 1969. Cell interaction in antibody synthesis. *J. Allergy.* **43**:323.

23. Hartmann, K., R. W. Dutton, M. M. McCarthy, and R. I. Mishell. 1970. Cell components in the immune response. II. Cell attachment separation of immune cells. *Cell. Immunol.* **1**:182.

24. Dutton, R. W., M. M. McCarthy, R. I. Mishell, and D. J. Raidt. 1970. Cell components in the immune response. IV. Relationships and possible interactions. *Cell. Immunol.* **1**:196.

25. Shortman, K., and J. Palmer. 1971. The requirements for macrophages in the *in vitro* immune response. *Cell. Immunol.* **2**:399.

26. Aaskov, J. G., and W. J. Halliday. 1971. Requirement for lymphocyte-macrophage interaction in the response of mouse spleen cultures to pneumococcal polysaccharide. *Cell. Immunol.* **2**:335.

27. Shortman, K., E. Diener, P. Russell, and W. D. Armstrong. 1970. The role of non-lymphoid accessory cells in the immune response to different antigens. *J. Exp. Med.* **131**:461.

28. Gordon, J., and L. D. MacLean. 1965. A lymphocyte-stimulating factor produced *in vitro*. *Nature (Lond.)* **208**:795.

29. Hoffmann, M., and R. W. Dutton. 1971. Immune response restoration with macrophage culture supernatants. *Science (Wash. D.C.)* **172**:1047.

30. Cruchaud, A., J. P. Despont, J. P. Girard, and B. Mach. 1970. Immunogenicity of human γ-globulin bound to macrophages after inhibition of RNA synthesis. *J. Immunol.* **104**:1256.

31. Roelants, G. E., and J. W. Goodman. 1969. The chemical nature of macrophage RNA-antigen complexes and their relevance to immune induction. *J. Exp. Med.* **130**:557.

32. Unanue, E. R., B. A. Askonas, and A. C. Allison. 1969. A role of macrophages in the stimulation of immune responses by adjuvants. *J. Immunol.* **103**:71.

33. Schmidtke, J., and E. R. Unanue. 1971. Interaction of macrophages and lymphocytes with surface immunoglobulin. *Nature (New Biol.) (Lond.)* **233**:84.

34. Campbell, D. A., and J. S. Garvey. 1961. The fate of foreign antigen and speculations as to its role in immune mechanisms. *Lab. Invest.* **10**:1126.

35. Rittenberg, M. B., and E. L. Nelson. 1960. Macrophages, nucleic acids, and the induction of antibody formation. *Am. Nat.* **94**:321.
36. Askonas, B. A., and J. M. Rhodes. 1965. Immunogenicity of antigen-containing ribonucleic acid preparations from macrophages. *Nature (Lond.)*. 205:470.

37. Fishman, M. 1961. Antibody formation in vitro. *J. Exp. Med.* 114:837.

38. Bishop, D. C., and A. A. Gottlieb. 1970. Macrophages, RNAs and the immune response. *Curr. Top. Microbiol. Immunol.* 51:1.

39. Paraf, A. 1970. Mécanisme d'action des adjuvants de l'immunité. *Ann. Inst. Pasteur (Paris).* 118:419.

40. Dresser, D. W., R. N. Taub, and A. R. Krantz. 1970. The effect of localized injection of adjuvant material on the draining lymph node. *Immunology.* 18:663.

41. Zatz, M. M., and E. M. Lance. 1971. The distribution of 51Cr-labeled lymphocytes into antigen-stimulated mice. Lymphocyte trapping. *J. Exp. Med.* 134:224.

42. Taub, R. N., and R. K. Gershon. 1972. The effect of localized injection of adjuvant material on the draining lymph node. III. Thymus dependence. *J. Immunol.* 108:377.

43. Ortiz-Ortiz, L., and B. N. Jaroslow. 1970. Enhancement by the adjuvant, endotoxin, of an immune response induced in vitro. *Immunology.* 19:387.

44. Thorbecke, G. J., and B. Benacerraf. 1962. The reticulo-endothelial system and immunological phenomena. *Prog. Allergy.* 6:559.

45. Schwartz, R. S., R. J. W. Ryder, and A. A. Gottlieb. 1970. Macrophages and antibody synthesis. *Prog. Allergy.* 14:81.

46. Abdou, N. I., and M. Richter. 1970. The role of bone marrow in the immune response. *Adv. Immunol.* 12:201.

47. Frei, P. C., B. Benacerraf, and G. J. Thorbecke. 1966. Phagocytosis of the antigen a crucial step in the induction of the primary response. *Proc. Natl. Acad. Sci. U.S.A.* 53:20.

48. Schmidtke, J. R., and E. R. Unanue. 1971. Macrophage-antigen interaction. Uptake, metabolism and immunogenicity of foreign albumin. *J. Immunol.* 107:331.

49. Galliley, R., and M. Feldman. 1966. The induction of antibody production in X-irradiated animals by macrophages that interacted with antigen. *Isr. J. Med. Sci.* 2:358.

50. Pinckard, R. N., D. M. Weir, and W. H. McBride. 1967. Factors influencing the immune response. I. Effects of the physical state of the antigen and of lympho-reticular cell proliferation on the response to intravenous injection of bovine serum albumin in rabbits. *Clin. Exp. Immunol.* 2:331.

51. Dresser, D. W. 1968. An assay for adjuvant activity. *Clin. Exp. Immunol.* 3:877.

52. Spitznagel, J. K., and A. C. Allison. 1970. Mode of action of adjuvants. Retinol and other lysosome-activating agents as adjuvants. *J. Immunol.* 104:119.

53. Weissmann, G., and P. Dukor. 1970. The role of lysosomes in immune responses. *Adv. Immunol.* 12:283.

54. Haskell, J. S., J. Marbrook, and B. E. Elliott. 1971. Thymus-independent step in the immune response to sheep erythrocytes. *Nature (New Biol.) (Lond.)*. 233:237.

55. Martial-Lasfargues, C., M. Liacopoulos-Briot, and B. N. Halpern. 1967. Culture des leucocytes sanguins de souris in vitro. Etude de l'action de la phytohémagglutinine (PIHA) sur les lymphocytes de souris normales et thymectomisées. *C. R. Soc. Biol.* 160:2013.
56. Greaves, M. F., I. M. Roitt, and M. E. Rose. 1968. Effect of bursectomy and thymectomy on the responses of chicken peripheral blood lymphocytes to phytohaemagglutinin. *Nature (Lond.)* 220:293.

57. Doenhoff, M. J., A. J. S. Davies, E. Leuchars, and V. Wallis. 1970. The thymus and circulating lymphocytes of mice. *Proc. Roy. Soc. Lond. B Biol. Sci.* 176:69.

58. Stobo, J. D., A. S. Rosenthal, and W. E. Paul. 1972. Functional heterogeneity of murine lymphoid cells. I. Responsiveness to and surface binding of concanavalin A and phytohemagglutinin. *J. Immunol.* 108:1.

59. Andersson, J., G. Möller, and O. Sjöberg. 1972. Selective induction of DNA synthesis in T and B lymphocytes. *Cell. Immunol.* In press.

60. Mooney, J. J., and B. H. Waksman. 1970. Activation of normal rabbit macrophage monolayers by supernatants of antigen-stimulated lymphocytes. *J. Immunol.* 105:1138.

61. Junge, U., J. Hockstra, and F. Deinhardt. 1971. Stimulation of peripheral lymphocytes by allogenic and autochthonous mononucleosis lymphocyte cell lines. *J. Immunol.* 106:1306.

62. Nathan, C. F., M. L. Karnovsky, and J. R. David. 1971. Alterations of macrophage functions by mediators from lymphocytes. *J. Exp. Med.* 133:1356.

63. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1971. Carrier function in anti-hapten 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.

64. Moore, R. D., and M. D. Schoenberg. 1971. Synthesis of antibody by lymphocytes restimulated in vitro with antigen. *J. Reticuloendothel. Soc.* 9:254.

65. Landy, M., and L. N. Chessin. 1969. The effect of plant mitogens on humoral and cellular immune responses. *Antibiot. Chemother.* 15:199.

66. Naspitz, C. K., and M. Richter. 1968. The action of phytohemagglutinin in vivo and in vitro, a review. *Prog. Allergy.* 12:1.

67. Peavey, D. L., W. H. Adler, and R. T. Smith. 1970. The mitogenic effects of endotoxin and staphylococcal enterotoxin B on mouse spleen cells and human peripheral lymphocytes. *J. Immunol.* 105:1453.