THE IN VITRO EFFECTS OF *BORDETELLA PERTUSSIS* LYMPHOCYTOSIS-PROMOTING FACTOR ON MURINE LYMPHOCYTES

II. Nature of the Responding Cells*

By AH SWEE KONG and STEPHEN I. MORSE

(From the Department of Microbiology and Immunology, State University of New York, Downstate Medical Center, Brooklyn, New York 11203)

In an accompanying communication (1) we described the in vitro mitogenic effect of the lymphocytosis-promoting factor (LPF)[1] of phase I *Bordetella pertussis* on murine lymphocytes. It was found that LPF caused proliferation of spleen and lymph node cells of unsensitized mice, but had no effect on bone marrow cells. The majority of thymocytes were not stimulated by LPF, but the population of cortisone-resistant thymocytes was reactive.

In this paper we present the results of experiments designed to delineate the nature of the lymphocyte population which responds to LPF, and the requirement for accessory cells in the attainment of an optimum mitogenic effect.

Materials and Methods

Mice. The strains of mice employed were as previously noted (1). In addition, congenitally athymic "nude" mice (*nu+/nu+*) were supplied by the Central Animal Service of the Downstate Medical Center. The mice were originally obtained from the Memorial-Sloan Kettering Cancer Center, New York, and have a BALB/c background.

Cell Cultures. The methods of cell preparation and culture, estimation of DNA synthesis, and the mitogens used have been described (1).

Thy-1.2 Antiserum. This reagent was prepared according to the method of Reif and Allen (2). AKR mice received six weekly intraperitoneal injections of 10×10^6 CBA thymus cells, following which serum was obtained and heated at 56°C for 30 min before use. For lysis of sensitive cells, CBA spleen cells at a concentration of 25×10^6 cells/ml were incubated in an appropriate dilution of the antiserum for 60 min at 37°C. The cells were then washed once and resuspended in a 1/8 dilution of guinea pig serum (Grand Island Biological Co., Grand Island, N. Y.) which had previously been absorbed in the cold with CBA thymocytes. After further incubation at 37°C for 30 min, the cell suspension was centrifuged, the cells washed once, and the residual number of viable

* This study was supported in part by Grant AI 09683 from the National Institutes of Health, U. S. Public Health Service. Portions of this work were submitted by A. S. K. in partial fulfillment of the requirements for the Ph.D. degree in the Department of Microbiology and Immunology, Downstate Medical Center.

[1] *Abbreviations used in this paper*: Con A, concanavalin A; FCS, fetal calf serum; LPF, lymphocytosis-promoting factor; LPS, lipopolysaccharide endotoxin isolated from *Salmonella typhosa* by the phenol-water technique; 2-ME, 2-mercaptoethanol; NBCS, new-born calf serum; PHA, phytohemagglutinin; RPMI-1640, Roswell Park Memorial Institute 1640 medium.

cells determined using the trypan blue dye exclusion test. Heat-inactivated normal AKR serum was used as a control for the Thy-1.2 antiserum.

Separation of Adherent from Nonadherent Cells. Spleen cells were fractionated on nylon wool columns by the methods of Julius et al. (3) and Handwerger and Schwartz (4). In brief, 1.2 g of prewashed nylon wool (FT-242, Fenwall Laboratories, Inc., Morton Grove, Ill.) was packed into the barrel of a 20-ml disposable plastic syringe. After autoclaving, the column was prerinsed with 50 ml of RPMI 1640-10% fetal calf serum (FCS) and then incubated at 37°C for 1 h. 2 ml of RPMI-10% FCS containing 300×10^6 spleen cells was loaded on the column followed by 2 ml of medium. After incubating the column for 45 min in a 37°C humidified incubator with a gas phase of 5% CO_2 in air, the nonadherent cells were collected in a vol of 15 ml of medium which was added at a flow rate of 1 ml/2–3 min. The column was then washed with a large (100 ml) volume of medium at 37°C, residual medium was expressed by compression with the syringe plunger, and the adherent cells detached from the nylon wool by five cycles of teasing with forceps in 10 ml of medium at 37°C.

Peritoneal Macrophages. Mice were killed by cervical dislocation and the abdominal skin reflected. 1.5 ml of RPMI 1640 containing heparin was injected intraperitoneally and the fluid withdrawn with a Pasteur pipette. The number of viable cells was enumerated and the cells resuspended in RPMI 1640-20% new born calf serum (NBCS, Microbiological Associates, Bethesda, Md.) to a concentration of 2×10^6 cells/ml. 25-ml aliquots of the suspension were dispensed to glass Petri dishes (100 mm × 15 mm). After incubation in a CO_2 incubator at 37°C for 60 min, the nonadherent cells were removed and the adherent layer washed twice with 10 ml of RPMI 1640-20% NBCS. The dishes containing the adherent cells were cultured for 5 days, with daily changing of the culture medium. At the end of this time period the cells were scraped free with a rubber policeman.

Reagents and Chemicals. Methyl-α-D-*N*-acetyl mannoside was obtained from Sigma Chemical Co., St. Louis, Mo., and *N*-acetyl-D-galactosamine from Schwarz/Mann, Orangeburg, N. Y.

Electron Microscopy. Cell pellets were fixed with glutaraldehyde and post-fixed in osmium before embedding in Araldite. Specimens were examined in a Siemens 1A electron microscope.

Results

The Effect of LPF on Spleen Cells of Nude Mice. The inability of LPF to stimulate bone marrow cells (1) suggested that T cells were required for proliferation. It was therefore of interest to determine whether LPF would initiate a mitogenic response in spleen cells of congenitally athymic nude mice which lack mature T cells (5). As can be seen in Table I, LPF stimulated spleen cells from normal BALB mice but not spleen cells from nude mice. Similarly, PHA, a murine T-cell mitogen (6), stimulated only normal spleen cells. In contrast LPS, a specific mitogen for murine B cells (7), induced proliferation of spleen cells from both nude and normal mice. The lack of in vitro stimulation of spleen cells from nude mice by LPF stands in marked contrast to the ability of LPF to induce lymphocytosis in these animals (8).

The Effect of Thy-1 Antiserum on the Initiation of the Response to LPF. Another method for testing for the requirement of T cells, in addition to examining the effects of LPF on bone marrow cells and spleen cells from nude mice, was to employ Thy-1 antiserum. Thy-1 antigen (theta antigen) is present on the surface of mouse thymocytes and peripheral T cells (9). In the case of CBA mice the Thy-1.2 alloantigen is found. Therefore, CBA spleen cells were first incubated with Thy-1.2 antiserum and a source of complement, and then the effect of LPF on cultures of the residual viable cells was tested. The results presented in Table II demonstrate that LPF, like phytohemagglutinin (PHA), did not stimulate spleen cells which were insusceptible to the action of Thy-1.2 antiserum.

The Effect of Thy-1.2 Antiserum on Proliferating Cells. The above studies

TABLE I

[^{3}H]thymidine Incorporation by Spleen Cells from Nude Mice in Response to Mitogens

Cell Source	Mitogen	[^{3}H]thymidine incorporation*
Normal BALB spleen	None	5,445 ± 327
	PHA (2.5 μl)	46,994 ± 2,603
	LPS (5.0 μg)	41,456 ± 2,626
	LPF (0.125 μg)	53,209 ± 24,852
	LPF (0.25 μg)	117,264 ± 12,232
	LPF (0.5 μg)	113,567 ± 4,377
"Nude" spleen	None	1,375 ± 598
	PHA (2.5 μl)	1,069 ± 220
	LPS (5.0 μg)	51,492 ± 3,135
	LPF (0.125 μg)	260 ± 71
	LPF (0.25 μg)	500 ± 41
	LPF (0.5 μg)	918 ± 38

* Mean counts per minute ± SD of triplicate cultures containing 0.5 × 10^6 cells/0.25 ml at 72 h.

TABLE II

Effect of Treatment with Thy-1.2 Antiserum on the Responsiveness of CBA Spleen Cells

Treatment of the Cells	Mitogen		
	None	PHA (2.5 μl)	LPF (0.5 μg)
None	8,647 ± 1,003*	65,314 ± 1,725	145,587 ± 7,211
Normal mouse serum + C	1,926 ± 710	68,639 ± 1,156	122,536 ± 2,050
Thy-1.2 antiserum + C	3,854 ± 1,597	1,729 ± 176	2,523 ± 287

* Mean counts per minute ± SD at 72 h of cultures containing 0.5 × 10^6 cells/0.25 ml.

clearly demonstrated that T cells were required for the proliferative response to LPF to take place, but did not prove that the cells that were proliferating were actually T cells. To determine this, the effect of Thy-1 antiserum on stimulated cells was examined. CBA spleen cells were first cultured with an optimum amount of LPF for 64 h and then triplicate cultures were treated with either Thy-1.2 antiserum or normal mouse serum, plus complement, or left untreated. Then, [^{3}H]thymidine was added and the cultures harvested 8 h later.

Cultures that were treated in this fashion with Thy-1.2 antiserum had markedly diminished [^{3}H]thymidine incorporation (Table III), whereas normal mouse serum had no effect. Thus, it appeared that not only were T cells required for the response, but they were also the responding cells.

Electron Microscopical Appearance of Spleen Cells Stimulated by LPF. Janossy et al. (10) have depicted the ultrastructural features of murine T and B cells stimulated by mitogens in vitro. Activated cells of both types exhibit: larger size with increased cytoplasmic/nuclear ratio; loosely arranged euchromatic with clumps of dense heterochromatin near the nuclear membrane; numerous pale, round, swollen mitochondria; well-developed Golgi apparatus; endocytic vesicles, multivesicular bodies and dense bodies; prominent areas containing pale, round, particles presumed to be glycogen; and either numerous

TABLE III
Effect of Treatment with Thy-1.2 Antiserum and Complement on LPF-Stimulated CBA Blast Cells

Treatment of the cells*	Percent lysis	[^{3}H]thymidine incorporation
None	0	49,949 ± 3,899
Normal mouse serum + C	17.4	55,787 ± 448
Thy-1.2 antiserum + C	88.1	3,968 ± 70

* Spleen cell cultures containing 0.5 × 10^6 cells and 0.5 μg of LPF in 0.25 ml were treated at 64 h with (1:10) anti-Thy 1.2 and complement (C) or normal mouse serum + C. [^{3}H]thymidine was added and the incorporation expressed as the mean cpm ± SD of triplicate cultures 8 h later.

single ribosomes or polysomes. As shown in Fig. 1, these were characteristic of CBA spleen cells which had been incubated with LPF for 3 days.

The central feature which distinguishes between stimulated T and B cells is that activated murine B cells contain dilated cisternae of rough endoplasmic, whereas T-cell blasts possess only flattened, undilated rough endoplasmic reticulum (10). In the case of LPF, the stimulated cells lacked dilated rough endoplasmic reticulum (Fig. 1) and therefore resembled T-cell rather than B-cell lymphoblasts.

Comparison of T-Cell Populations Activated by LPF, Concanavalin A (Con A), and PHA. It was clear that the spectrum of activity of LPF differed from that of the T-cell mitogen Con A, since Con A stimulated normal thymocytes whereas LPF, like PHA, activated only cortisone-resistant thymocytes (1). However, the available data indicated that LPF might affect the same T cells as PHA.

Heiniger et al. (11) analysed the response of peripheral blood lymphocytes of 59 inbred strains of mice to PHA and found that the family of DBA mice responded least. Similarly, Dumont (12) reported that DBA/2 spleen cells exhibited only marginal stimulation by PHA, although they did respond fully to the B-cell mitogen LPS. We therefore examined the in vitro effects of LPF and Con A, as well as PHA, on cultures of spleen cells from DBA/2J mice in order to determine whether these cells were refractory to all three T-cell mitogens.

It can be seen in Fig. 2 that DBA/2J spleen cells did not respond to PHA, and this was true over a wide dose range of the mitogen. However, DBA/2J spleen cells were markedly stimulated by LPF and Con A. Thus, LPF and PHA are not identical with respect to the T-cell populations which they can activate.

Responsiveness to PHA and Con A have been shown to be specifically inhibited by simple monosaccharides. *N*-acetyl-D-galactosamine will inhibit stimulation by PHA (13), while α-methyl-D-mannoside inhibits Con A (14). Although a complete search for simple compounds capable of inhibiting LPF has not been undertaken, it was of interest to determine whether these two substances were inhibitory. As shown in Table IV, neither acetylgalactosamine nor the mannoside had any effect on LPF.

Requirements for Accessory Cells. Although Thy-1.2 susceptible T cells were shown to be required for the initiation of the response to LPF and, in addition,

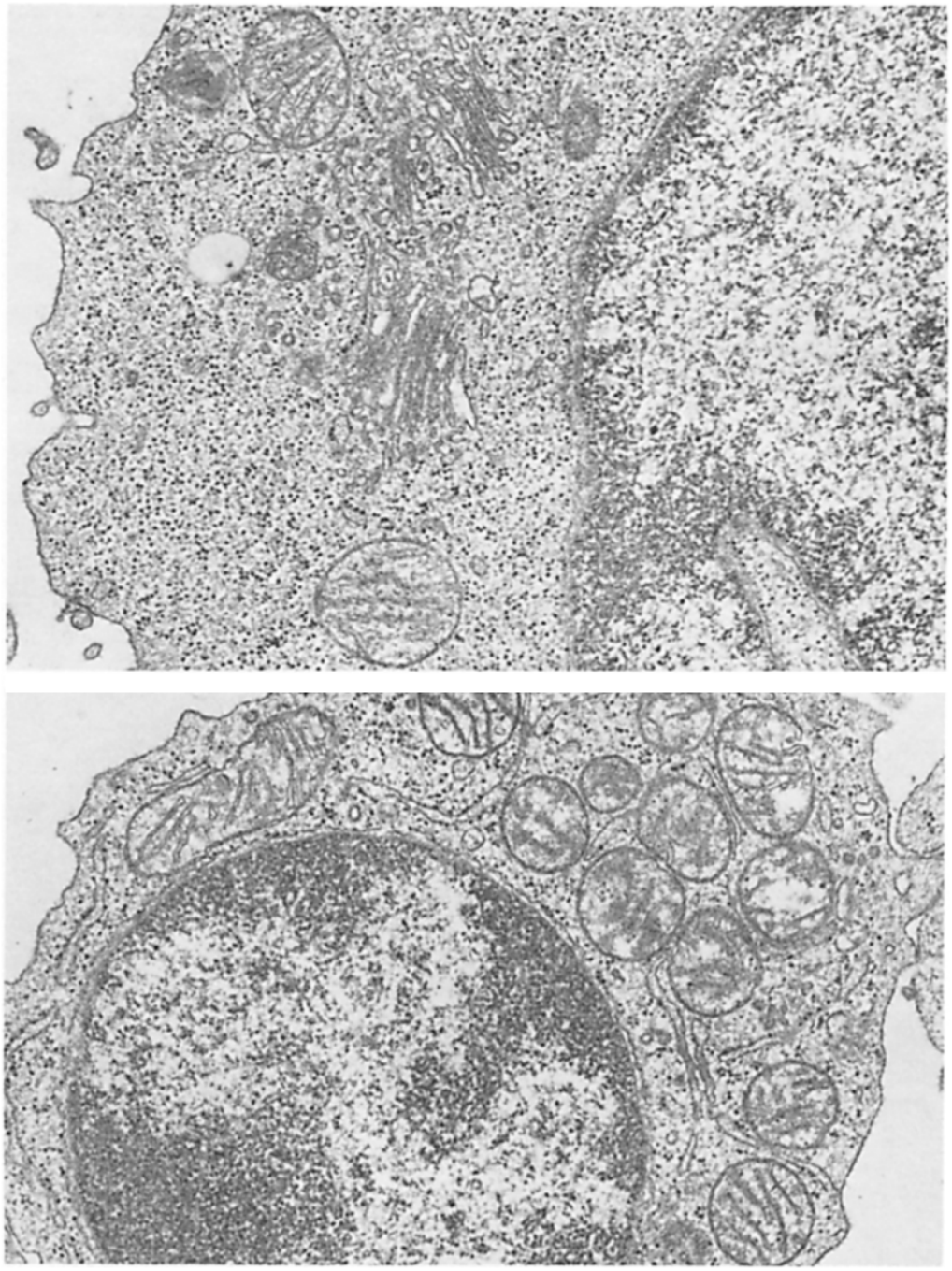

FIG. 1. Electron microscopic appearance of lymphoblasts in CBA spleen cell cultures incubated with LPF for 72 h. (× 25,000).

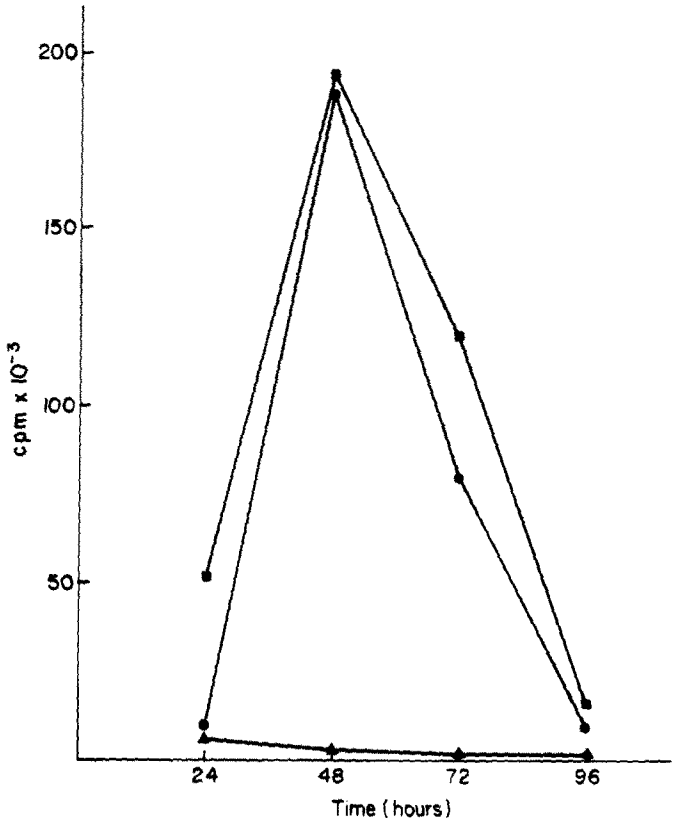

FIG. 2. Response of 0.5×10^6 spleen cells from DBA/2J mice cultured for various time periods in the presence of 2.5 μl of PHA (▲–▲), 0.5 μg of Con A (■–■), or 0.5 μg of LPF (●–●).

comprised the population of cells incorporating [^{3}H]-thymidine, it was possible that another cell type was necessary for the reaction to proceed optimally.

Adherent and nonadherent spleen cells were separated on nylon wool columns and individually tested at cell concentrations of 0.5×10^6/0.25 ml for stimulation by LPF, Con A, PHA, and lipopolysaccharide (LPS). The responses were compared with those attained by unseparated spleen cells. As can be seen in Table V, the nonadherent cells, which consist primarily of T cells (3, 4), gave as expected, a full response to PHA and were markedly stimulated by Con A. In contrast, LPS, a B-cell mitogen stimulated only the B-cell-enriched adherent cells (3, 4).

It might be predicted that the nonadherent population would respond to LPF in a manner similar to that seen with the T-cell mitogens, PHA and Con A. However, the responses to both 0.25 and 0.5 μg of LPF were markedly decreased in the separated populations when compared with the responses of nonseparated cells. The minimal response of the nonadherent cells was completely abolished when the cells were subjected to a second column treatment to remove residual adherent cells.

The effect of admixture of adherent and nonadherent cells on responsiveness

TABLE IV
Inhibition of Mitogenic Activity by Sugars

Mitogen	Percent inhibition of [^{3}H]thymidine incorporation by			
	NAGAL*		MAM*	
	0.5 mg‡	1.5 mg	0.5 mg	1.5 mg
None	0	0	0	0
Con A	5	4	0	98
PHA	0	45	0	4
LPF	0	1	0	0

* NAGAL, *N*-acetyl-D-galactosamine; MAM, α-methyl-D-mannoside.
‡ Amount in 0.25 ml containing 0.5 × 10^6 CBA spleen cells and 0.5 μg Con A, 2.5 μl of PHA, or 0.5 μg of LPF. [^{3}H]thymidine incorporation was assayed at 72 h.

TABLE V
Response of Nylon-Wool Separated CBA Spleen Cell Populations to Mitogens

Mitogen	[^{3}H]thymidine incorporation*		
	Unseparated	Adherent	Nonadherent
Con A (0.5 μg)	120,162 ± 10,118	14,962 ± 1,088	87,750 ± 14,499
PHA (2.5 μg)	49,138 ± 5,201	2,633 ± 642	40,365 ± 11,030
LPF (0.25 μg)	93,116 ± 5,012	5,140 ± 826	4,102 ± 542
LPF (0.5 μg)	146,547 ± 4,349	9,592 ± 3,989	31,482 ± 5,357
LPS (5.0 μg)	83,593 ± 7,956	70,388 ± 14,942	930 ± 294
None	3,036 ± 288	1,430 ± 370	102 ± 6

* Mean counts per minute ± SD of triplicate cultures containing 0.5 × 10^6 cells/0.25 ml at 72 h.

to LPF was then examined, keeping the total number of cells constant at 0.5 × 10^6/0.25 ml. In Fig. 3 it is shown that striking synergy between the two populations occurred over a wide range of ratios of adherent:nonadherent cells (1:3–3:1).

The nature of the adherent cell type which synergized with the nonadherent cells was next examined. It was first determined that adherent cells, both in the presence and absence of LPF, did not release soluble factors which stimulated the nonadherent cells, indicating that cell-to-cell contact, or at least close approximation, was required for synergy. Treatment of adherent CBA spleen cells with Thy-1 antiserum did not affect their synergizing capacity, indicating that an adherent T-cell population was not responsible.

Macrophages, which are present in the adherent cell population, have been implicated as required accessory cells for murine T-cell activation by antigen and in mixed lymphocyte reactions (15), but are apparently not necessary for stimulation by PHA or Con A (4, 16). However, there is considerable controversy as to their role in mitogen activation in other species (17–22). To determine whether macrophages played a role in LPF activation of murine T cells, unseparated CBA spleen cells, as well as the separated adherent cells, were depleted of macrophages by allowing the phagocytic cells to ingest carbonyl iron and then removing them by passing the cell suspensions through a strong magnetic field (23). Such treatment neither reduced the responsiveness of CBA spleen cells to

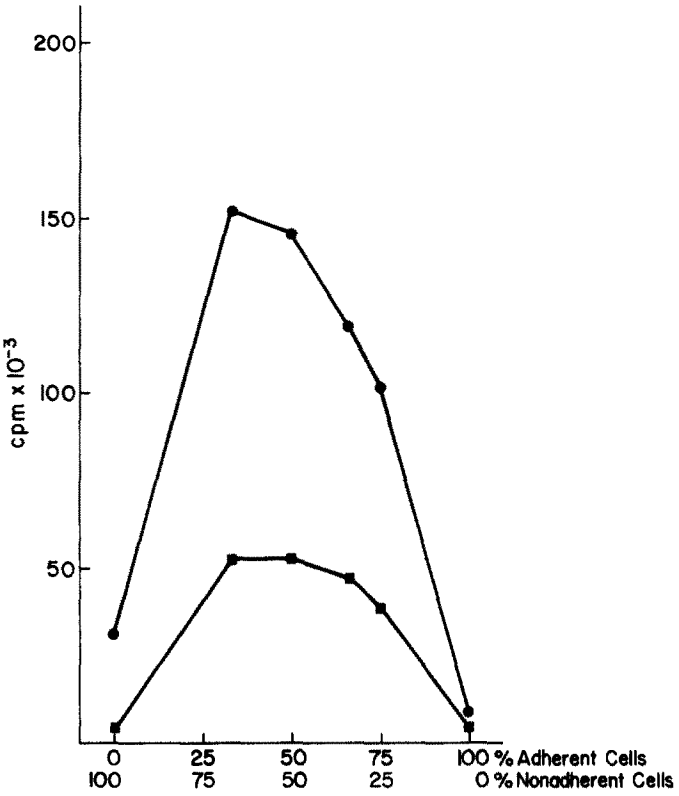

FIG. 3. Synergy in the proliferative response to 0.25 μg (■ – ■) and 0.5 μg (● – ●) of LPF. Cultures contained various proportions of adherent and nonadherent CBA spleen cells as indicated, and the total number of cells was 0.5 × 10^6. The data are expressed as the mean counts per minute of triplicate cultures less the mean counts per minute of control cultures at 72 h.

LPF nor abrogated the synergistic effect of the nylon-wool adherent cells. Further evidence that macrophages were not vital accessory cells was obtained when it was found that mouse peritoneal macrophages did not restore the responsiveness to LPF of the nonadherent spleen cell population (Table VI).

2-mercaptoethanol (2-ME) promotes the viability of lymphoid cells in culture and thereby increases their responsiveness (24, 25). However, the addition of 2-ME cultured nonadherent CBA spleen cells did not result in stimulation by LPF (Table VII).

Discussion

The LPF from phase I *Bordetella pertussis* is a potent mitogen for cultured murine lymphoid cells (8), and this study was designed to delineate the nature of the responding cells. Previously, it was shown that cells from spleens and lymph nodes, which contain both T and B cells, responded to LPF, whereas bone marrow cells, which are T-cell deficient, did not undergo proliferation. This phenomenon suggested LPF is probably a T-cell mitogen and not a B-cell mitogen. The hypothesis was further supported by the finding that spleen cell

TABLE VI
Effect of Peritoneal Macrophages on the Proliferative Response of Nonadherent CBA Spleen Cells

Spleen cells	Percent macrophages added	LPF	
		None	0.5 μg
Unseparated	0	4,997 ± 1,801*	173,286 ± 10,185
Nonadherent	0	751 ± 96	18,951 ± 717
	1	3,862 ± 502	30,835 ± 4,376
	5	2,959 ± 386	28,816 ± 1,987
	10	3,017 ± 668	21,480 ± 2,237
	15	3,703 ± 376	17,375 ± 1,331
	20	2,907 ± 336	11,263 ± 203

* Mean counts per minute ± SD of triplicate cultures containing 0.5×10^6 cells/0.25 ml at 72 h.

TABLE VII
Effect of 2-Mercaptoethanol on the Proliferative Response of Nonadherent CBA Spleen Cells

Spleen cells	2-ME (μM)	LPF	
		None	0.5 μg
Unseparated	0	3,336 ± 506*	152,862 ± 14,415
Nonadherent	0	865 ± 163	50,710 ± 2,399
	10	216 ± 88	34,224 ± 2,103
	20	731 ± 14	45,523 ± 2,367
	40	1,033 ± 228	45,504 ± 660
	60	821 ± 146	36,270 ± 182

* Mean counts per minute ± SD of triplicate cultures containing 0.5×10^6 cells/0.25 ml at 72 h.

suspensions from congenitally athymic nude mice which lack mature T cells (5) also did not respond to LPF.

Critical evidence that LPF is a murine T-cell mitogen was derived from studies on the effect of Thy-1.2 antiserum which reacts with thymus and peripheral T cells. In the presence of complement, T cells which possess the Thy-1.2 alloantigen by the antiserum are lysed and CBA spleen cell suspensions treated in this manner no longer responded to LPF, thereby indicating that T cells were required for LPF activation. However, this type of experimental approach did not demonstrate whether T cells were required accessory cells or were the reactive cells.

Two independent types of evidence were adduced to show that the proliferating lymphoid cells were T cells. CBA spleen cells which were cultured with LPF for 64 h were treated with Thy-1.2 antiserum plus complement just before the addition of [^{3}H]thymidine. 8 h later the cells were harvested, and it was found that the residual viable cells had not incorporated tritiated thymidine. Thus, the activated cells possessed the T-cell antigen Thy-1.2 on their surface. Moreover, the electron microscopic appearance of LPF-induced lymphoblasts was that of T-cell blasts (10). In particular, dilated rough endoplasmic reticulum which is characteristic of B-cell blasts (plasmablasts) was absent.

There is abundant evidence that specificity of mitogens for T or B lymphocytes is unrelated to specific binding. For example, it appears that the T-cell mitogens, PHA and Con A, and the B-cell mitogen, LPS, all bind equally well to both cell types (26–28). Studies utilizing ^{125}I-labeled material will provide information as to whether LPF has any binding specificity. It will also be of interest to determine whether altering the physical state of LPF affects the selectivity of LPF for T cells as occurs with PHA and Con A which when insolubilized stimulate B cells as well as T cells (29, 30).

There is increasing evidence for functional heterogeneity and subsets among T lymphocytes, and the finding of differences in populations responding to different T-cell mitogens is an important key in elucidating the nature of the heterogeneity of T cells. In this regard, LPF may prove to be a useful substance since it has a different spectrum of activity from either Con A or PHA. LPF is distinguished from Con A because only cortisone-resistant thymocytes are stimulated by LPF, whereas both cortisone-sensitive and cortisone-resistant cells respond to Con A. PHA and LPF do not affect an identical T-cell population, since spleen cells from DBA/2J mice are insensitive to PHA but fully activated by LPF. The reasons for these differences remain to be explained although the receptors appear to be different. It will be important to determine whether any other functional properties are specifically associated with the T-cell population responsive to LPF.

LPF activation of T cells clearly requires a "non T cell" for optimal proliferation. When spleen cells which did not absorb to nylon wool columns were incubated with LPF only minimal mitogenic stimulation was found; after a second column treatment the incorporation of [^{3}H]thymidine was reduced to background levels. The nonadherent cells obtained by this procedure are enriched in T cells, and this was evidenced by their responsiveness to the T-cell mitogens Con A and PHA, even though they did not respond to LPF. The observation that Con A and PHA produced activation of nonadherent spleen cells, whereas LPF did not, also points to differences in the action of these T-cell mitogens.

Adherent cells are enriched in both B cells and phagocytic cells, particularly mononuclear phagocytes, and this population was only minimally stimulated by Con A, PHA, and LPF, but was fully responsive to the B-cell mitogen LPS.

As shown in Fig. 2, mixtures of the adherent and nonadherent populations were stimulated by LPF, suggesting that an accessory cell, or factor, found in or produced by the adherent population, was required for T-cell activation by LPF. It was unlikely that a soluble factor was responsible and although identification of the responsible adherent cell(s) has not been achieved, certain preliminary findings are worth noting. Firstly, an adherent T cell with surface Thy-1 antigen is not involved, since treatment of the adherent population with Thy-1.2 antiserum plus complement did not affect synergistic activity. It is difficult to entirely exclude a role for macrophages, but the lack of effect of removal of phagocytic cells by carbonyl iron treatment, and the inability of peritoneal macrophages to substitute for adherent cells and support LPF stimulation of nonadherent cells argue against this possibility. 2-ME, which enhances reactivity by increasing viability (24, 25), was also ineffective in restoring activity to the nonadherent population.

There is then the possibility that B cells are required for T-cell activation by LPF, although there is little precedent for this type of synergy. Alternatively, a cell type which is thus far unrecognized as a constituent of adherent murine spleen cell populations may be responsible.

Summary

The mitogenic response of murine lymphocytes to the lymphocytosis-promoting factor of *Bordetella pertussis* has been shown to be due to activation of T cells. The selectivity of responsiveness to LPF with respect to the population of T cells which is stimulated, differs from that of PHA as well as Con A, and the surface receptors are different. A population of adherent cells, which does not appear to consist of macrophages or other phagocytic cells, is required for the T-cell response.

Mrs. Carol D. Stearns and Mrs. Susan R. Goldsmith provided expert technical assistance and Mr. Dennis Ryan kindly performed the electron microscopy.

Received for publication 15 September 1976.

References

1. Kong, A. S., and S. I. Morse. 1976. The in vitro effects of *Bordetella pertussis* lymphocytosis-promoting factor on murine lymphocytes. I. Proliferative response. *J. Exp. Med.* 145:151.
2. Reif, A. E., and J. M. Allen. 1966. Mouse thymic isoantigens. *Natue (Lond.).* 209:521.
3. 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.
4. Handwerger, B. S., and R. H. Schwartz. 1974. Separation of murine lymphoid cells using nylon wool columns, *Transplantation (Baltimore).* 18:544.
5. Raff, M. D., and H. H. Wortis. 1970. Thymus dependence of θ-bearing cells in the peripheral lymphoid tissues of mice. *Immunology.* 18:931.
6. Janossy, G., and M. F. Greaves. 1971. Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. *Clin. Exp. Immunol.* 9:483.
7. Andersson, J., O. Sjöberg, and G. Möller. 1972. Induction of immunoglobulin and antibody synthesis *in vitro* by lipopolysaccharides. *Eur. J. Immunol.* 2:349.
8. Morse, S. I., and J. H. Morse. 1976. Isolation and properties of the leukocytosis- and lymphocytosis-promoting factor of *Bordetella pertussis*. *J. Exp. Med.* 143:1483.
9. Raff, M. C. 1971. Surface antigenic markers for distinguishing T and B lymphocytes in mice. *Transplant. Rev.* 6:52.
10. Janossy, G., M. Shohat, M. F. Greaves, and R. Dourmashkin. 1973. Lymphocyte activation. IV. The ultrastructural pattern of the response of mouse T and B cells to stimulation in vitro. *Immunology.* 24:211.
11. Heiniger, H., B. A. Taylor, E. J. Hards, and H. Neier. 1975. Heritability of the phytohemagglutinin responsiveness of lymphocytes and its relationship to leukemogenesis. *Cancer Res.* 35:825.
12. Dumont, F. R. 1976. Contrasting electrophoretic distribution of splenic lymphocytes in two H-2 identical high- and low-phytohemagglutinin-responder mouse strains. *Folia Biol.* 22:1.
13. Borberg, H., I. Yesner, B. Gesner, and R. Silber. 1968. The effect of *N*-acetyl-D-galactosamine and other sugars on the mitogenic activity and attachment of PHA to tonsil cells. *Blood.* 31:747.

14. Powell, A. E., and M. Leon. 1970. Reversible enteraction of human lymphocytes with the mitogen concanavalin A. *Exp. Cell. Res.* **62**:315.
15. Phillips, S. M., C. B. Carpenter, and J. P. Merrill. 1972. Cellular immunity in the mouse. I. *In vitro* lymphocyte reactivity. *Cell. Immunol.* **5**:235.
16. Rosenstreich, D. L., and J. J. Oppenheim. 1976. The role of macrophages in the activation of T and B lymphocytes *in vitro*. *In* Immunobiology of the Macrophage. D. S. Nelson, editor. Academic Press, Inc., New York. 161.
17. 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.
18. Folch, H., M. Yoshinaga, and B. H. Naksman. 1973. Regulation of lymphocyte responses *in vitro*. III. Inhibition by adherent cells of the T-lymphocyte response to phytohemagglutinin. *J. Immunol.* **110**:835.
19. Waldron, J. A., R. G. Horn, and A. S. Rosenthal. 1973. Antigen induced proliferation of guinea pig lymphocytes *in vitro*: obligatory role of macrophages in the recognition of antigen by immune T-lymphocytes *J. Immunol.* **111**:58.
20. Lohrmann, H. P., L. Novitous, and R. G. Graw. 1974. Cellular interactions in the proliferative response of human T and B lymphocytes to phytomitogens and allogeneic lymphocytes. *J. Exp. Med.* **139**:1553.
21. Lipsky, P. E., J. J. Ellner, and A. S. Rosenthal. 1976. Phytohemagglutinin-induced proliferation of guinea pig thymus-derived lymphocytes. I. Accessory cell dependence. *J. Immunol.* **116**:868.
22. Oppenheim, J. J., and D. L. Rosenstreich. 1976. Signals regulating *in vitro* activation of lymphocytes. *Prog. Allergy.* **20**:65.
23. Schlossman, S. F., and L. Hudson. 1973. Specific purification of lymphocyte populations on a digestible immunoabsorbent. *J. Immunol.* **110**:313.
24. Chen, C., and J. G. Hirsch. 1972. The effects of mercaptoethanol and of peritoneal macrophages on the antibody-forming capacity of nonadherent mouse spleen cells *in vitro*. *Science* (*Wash. D.C.*). **176**:60.
25. Fanger, M. W., D. A. Hart, J. V. Wells, and A. Nisonoff. 1970. Enhancement by reducing agents of the transformation of human and rabbit peripheral lymphocytes. *J. Immunol.* **105**:1043.
26. Andersson, J., O. Sjöberg, and G. Möller. 1972. Mitogens as probes for immunocyte activation and cellular cooperation. *Transplant. Rev.* **11**:131.
27. Greaves, M., and G. Janossy. 1972. Elicitation of selective T and B lymphocyte responses by cell surface binding ligands. *Transplant. Rev.* **11**:87.
28. Stobo, J. D. 1972. Phytohemagglutinin and concanavalin A: probes for murine T cell activation and differentiation. *Transplant. Rev.* **11**:60.
29. Andersson, J., G. M. Edelman, G. Möller, and O. Sjöberg. 1972. Activation of B lymphocytes by locally concentrated concanavalin A. *Eur. J. Immunol.* **2**:233.
30. Greaves, M. F., and S. Bauminger. 1972. Activation of T and B lymphocytes by insoluble phytomitogens. *Nature* (*Lond.*) **235**:67.