# THE MODULATION OF LYMPHOCYTE FUNCTIONS BY MOLECULES SECRETED BY MACROPHAGES*

## II. Conditions Leading to Increased Secretion

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Culture fluids rich in mononuclear phagocytes have a powerful effect on various in vitro reactions of lymphocytes (1-9). The culture fluids contain a mitogen for thymocytes and mature lymphocytes (1-4, 8). Also found are active principles which differentiate memory B cells into antibody-secreting cells and which increase helper activities of T lymphocytes (2, 3). In a previous report we obtained from experiments in which lymphocytes were depleted from the cell preparations, strong evidence that the cellular source of the active component(s) was the macrophage (3). The activity secreted into the medium, however, was variable from experiment to experiment, perhaps related to the state of macrophage activation. In this paper we present results of experiments in which we studied the relation between macrophage activation and/or stimulation with the secretion of the various activities. We have found two ways in which to generate high levels of lymphostimulatory activities in macrophage cultures: one is to add a series of materials that are readily taken up by the macrophages; the second is to add a small number of activated T cells to the macrophage-rich cultures. Other experiments indicate that there is an inverse relationship between macrophage activation and the secretion of the lymphostimulatory activities.

## Materials and Methods

*Culture Fluids.* Peritoneal exudate cells (PEC)[1] were harvested from mice, washed once, and cultured at an initial density of $4 \times 10^6$ cells per ml, usually, in 1640 medium containing 5% fetal calf serum (vol/vol) (3) in 35 × 10-mm dishes (Falcon Plastics, Division of Bioquest, Oxnard, Calif.). In all experiments the macrophages were planted for an initial period of 2 h, after which the dishes were shaken hard to remove loosely adherent cells. The dishes containing the adherent cells were washed and then cultured for various times, usually for 24-48 h. The PEC were obtained from A/St mice (West Seneca Laboratories, Buffalo, New York), about 8-12 wk of age, of either sex. The A/St mice were either untreated or had been injected intraperitoneally (i.p.) with one of the following: 1.5 ml of a 10% proteose peptone solution (Difco Laboratories, Detroit, Mich.); 1 ml of thioglycollate broth (Difco Laboratories, Catalog no. 0236-01); or 100 μg of *Escherichia coli* lipopolysaccharide (LPS) (Difco Laboratories, Westphal preparation). The doses used and time of

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[1] *Abbreviations used in this paper:* F, fluorescein; F.KLH, fluorescein-conjugated hemocyanin; F.RGG, fluorescein-conjugated rabbit IgG; PEC, peritoneal exudate cells; PEL, peritoneal exudate lymphocytes; PFC, plaque-forming cells; LPS, *E. coli* lipopolysaccharide.

harvesting, 3-days postinjection, gave rise to populations of PEC containing mostly macrophages activated by morphological criteria (i.e., large degree of spreading, abundant lysosomes, and pinocytic vesicles). A series of experiments was done using PEC from *Listeria monocytogenes*-infected mice. Mice received an i.p. dose of $2 \times 10^3$ organisms (LD 50, $2 \times 10^5$), 7 days later 1.5 ml of 10% peptone i.p. 3 days later, the mice were sacrificed and the PEC harvested (10 days after infection). These exudates were sterile and contained abundant activated macrophages. All culture fluids were dialyzed against culture medium for 72 h before testing.

*Assay for Lymphostimulatory Activities.* Two assays were done (3). In all experiments culture fluids were tested for mitogenic activity on thymocytes. Thymocytes ($5 \times 10^6$) were cultured in 1 ml of 1640 medium in 12 × 75-mm tubes for 72 h at 37°C in a 5% $CO_2$ in-air incubator (3). The cultures received 1 $\mu$Ci of [$^3$H]thymidine about 12 h before harvesting. Incorporation of labeled $^3$H into acid insoluble precipitate was measured by standard methods. The background counts per minute (cpm) of thymocytes ranged from 400 to 1,000 cpm, usually about 500. The macrophage culture fluids were mixed (vol/vol) with normal medium and assayed for their mitogenic principle on thymocytes as above. Frequently, phytohemagglutinin (PHA) was added to the cultures together with macrophage culture fluids (1).

The second assay used in most but not all experiments was to test for antibody responses, in vitro, using the method of Mishell and Dutton (10). Spleen cells from mice immunized to fluorescein (F)-conjugated hemocyanin (KLH) were cultured with 0.1 $\mu$g of F.KLH, 0.1 $\mu$g of F-conjugated rabbit IgG (RGG), or without antigen for 4 days in the presence or absence of the macrophage culture fluids. Anti-F-secreting cells (plaque-forming cells, PFC) were detected by a plaque method using F-conjugated red cells. Two effects of macrophage culture fluids were usually noted. One was an increase of PFC to F, in the absence of antigen challenge, an effect considered to be a B-cell differentiating activity (3). The second effect was a higher increase in PFC in the presence of F.KLH which we attribute to a combined effect of the active principle(s) on both B and T cells. Full details of methods and of our interpretation of the effects of macrophage fluids were outlined in our previous paper (3).

*Separation of T Cells.* T lymphocytes from PEC (peritoneal exudate lymphocytes, PEL) or from spleen were isolated by the method of Julius et al. (11). We first planted cells in culture dishes for 1 h ($10^7$ per ml) and passed the nonadherent population through nylon wool columns. The preparations consisted of about 98% T cells and were essentially devoid of macrophages and B cells. Only a very occasional macrophage was found upon 2 or 3 days of culture of the T-cell preparations. The T cells were cultured in 1640 medium, as described, usually with the addition of 2-mercaptoethanol ($10^{-4}$ M). Survival of cells in culture for the first 48 h was excellent (approximately 75%).

*Challenge of Macrophages.* We tested the effects of various materials on the production of the lymphostimulatory activities: *(a)* sheep red cells incubated with a subagglutinating dose of rabbit IgG antibody; dose of red cells to PEC was 10:1; *(b) Listeria monocytogenes* vaccine, 50 bacteria per PEC; *(c)* latex beads of 1.09 $\mu$m diameter (Dow Chemical Company, Midland, Mich.), 50 latex beads per macrophage; *(d)* LPS, 50 $\mu$g per ml; and *(e)* beryllium sulfate ($BeSO_4$), 0.1 $\mu$g per ml.

*Miscellaneous.* In one experiment PEC were treated with anti-$\theta$ antibodies and complement in order to kill any T cells adhering to dishes (3). The anti-$\theta$ antibodies were from the same batch used previously (3). The source of complement was guinea pig serum.

## Results

*Effect of Various Stimuli on the Production of Lymphostimulatory Activities.* PEC culture fluids stimulated DNA synthesis of thymocytes and an increased PFC response in immunized spleen cells. The activity of PEC cultures under basal conditions was variable and usually tended to be low (3). However, the addition to the macrophage culture of various agents, such as bacteria, antigen-antibody complexes, latex particles, etc., led to an abrupt but usually temporary increase in the activities. In the experiments shown in Figs. 1 and 2, peptone-induced PEC were planted in culture and challenged for 24 h with antibody-coated sheep red cells or *Listeria* vaccine. Fig. 1 shows that culture

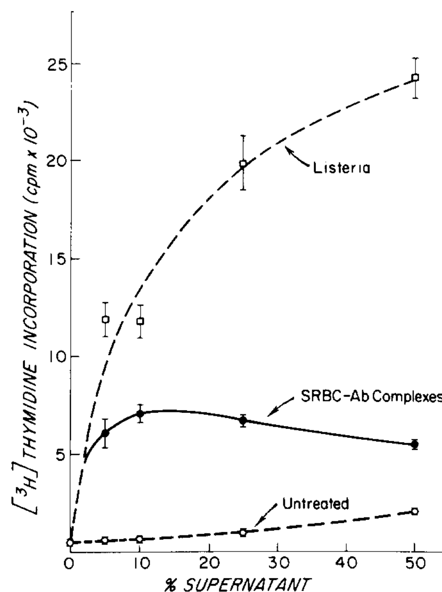


FIG. 1. The figure indicates the incorporation of radiolabeled thymidine by thymocytes cultured in PEC culture fluids after exposure to antibody-coated red cells or to *Listeria* vaccine. The PEC culture fluid was obtained during the first 24 h of culture.

fluids from treated PEC contained a higher amount of the mitogenic factor compared to cultures from untreated macrophages. The incorporation of [$^3$H]thymidine was increased about fivefold by fluids from PEC challenged with opsonized sheep cells and about 20-fold by fluids from macrophages challenged with *Listeria* organisms.

The effects of the culture fluids on antibody formation are depicted in Fig. 2. Spleen cells from mice immunized to F.KLH were cultured for 4 days with F.KLH, F.RGG, or without antigen in the presence or absence of the macrophage culture fluids. The following points can be made from this experiment: *(a)* as before (3), the macrophage fluids stimulated the development of PFC in the absence of antigen challenge (left-hand box, no antigen), but fluids from macrophages exposed to *Listeria* and antibody-coated red cells had higher activity than those from untreated macrophages; *(b)* all fluids increased the response to F.KLH but more markedly the fluids from stimulated PEC (middle box); and *(c)* the response to F.RGG (last vertical box) was comparable to that seen with lymphocytes cultured without antigen.

Not shown in Figs. 1 and 2 are control results testing culture fluids in which the dead *Listera* organsisms were incubated for 24 h with medium. Such culture fluids did not affect the lymphocytes' responses.

We explored the above phenomenon further in the experiment shown in Fig. 3 and Table I. Peptone-induced PEC were exposed to various agents and cultured for a period of 3 days, harvesting the medium every 24 h. There were increased amounts of mitogenic activity in cultures of PEC challenged with *Listeria* organisms, the increased activity decreasing after 48 h of culture. Latex beads were as stimulatory, the activity decaying also at 72 h. Antibody-coated sheep

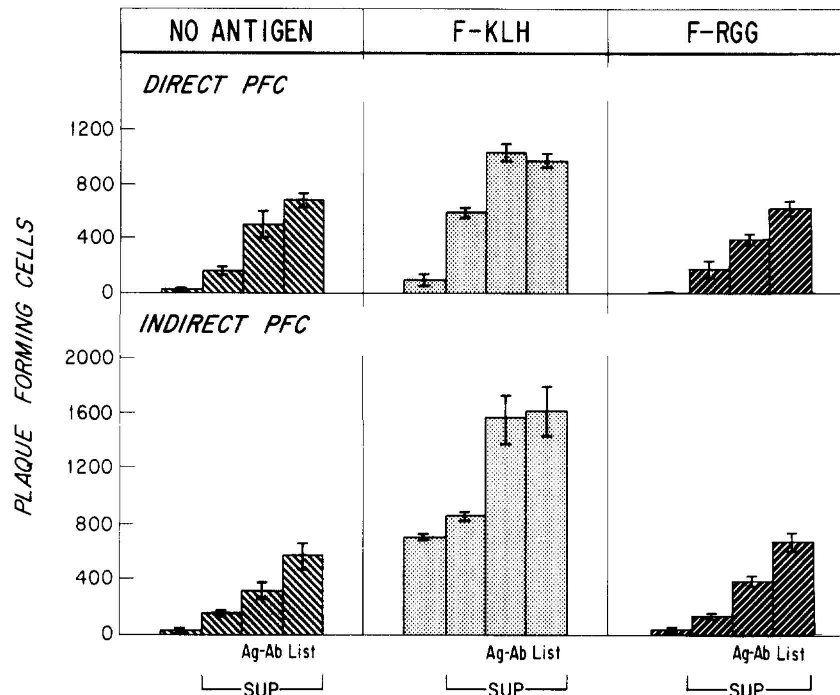


FIG. 2. The same PEC culture fluids of the experiment of Fig. 1 were tested on antibody formation in vitro. Ab-Ag refers to those PEC fluids obtained after uptake of antibody-coated red cells; list refers to fluids from PEC exposed to *Listeria*. The first vertical column in each box are cultures devoid of any PEC culture fluids. All cultures were tested at 25% concentration of PEC fluids.

TABLE I
*Effects of Different Macrophage Culture Fluids on the PFC Response*

| Addition to spleen cultures | No antigen | | F.KLH (0.1 μg) | |
|---|---|---|---|---|
| | IgM PFC | IgG PFC | IgM PFC | IgG PFC |
| No addition | 60 ± 0 | 53 ± 11 | 200 ± 40 | 203 ± 25 |
| 25% PEC fluids | | | | |
| Untreated PEC | 260 ± 40 | 293 ± 110 | 887 ± 75 | 1,180 ± 111 |
| After latex | 707 ± 70 | 527 ± 527 | 2,280 ± 485 | 2,093 ± 411 |
| After *Listeria* | 680 ± 69 | 527 ± 50 | 2,047 ± 121 | 2,633 ± 325 |
| After SRBC-antibody | 220 ± 53 | 200 ± 60 | 1,160 ± 69 | 1,373 ± 219 |
| After Be | 147 ± 31 | 160 ± 60 | 1,247 ± 133 | 1,740 ± 201 |
| After LPS | 300 ± 52 | 320 ± 35 | 1,440 ± 260 | 1,753 ± 500 |

IgM PFC refer to direct PFC; IgG are PFC detected with the use of facilitating antiserum. Each result is the mean PFC from two to three dishes (± standard error of the mean). Spleen cells were obtained from mice primed with F.KLH in Freund's adjuvant. The PEC culture fluids were obtained during the first 24-h period of culture. All PEC were from peptone-injected mice.

red cells were much less stimulatory. $BeSO_4$, a salt that stimulates macrophage activity in vitro (and which is also a potent adjuvant [12]), produced a slight increase in activity. Comparable results were obtained in antibody formation in vitro. We show in Table I only the results of fluids from the first 24 h of culture.

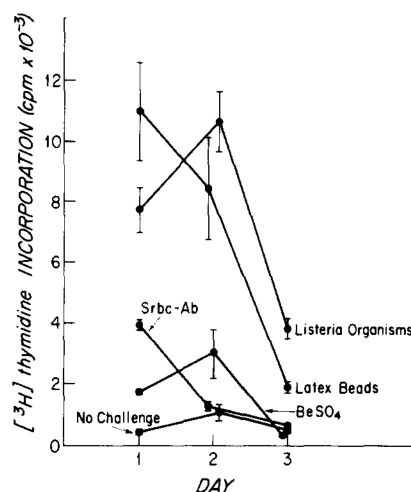


FIG. 3. PEC culture fluids were tested at a 25% concentration on thymocyte DNA synthesis. The explanation of the experiment is in the text.

The effects of LPS on the secretion were next examined. Peptone-induced PEC were exposed to LPS for 24 h. The culture fluids obtained during 24-h periods of culture were tested on the thymocyte DNA synthesis assay. A representative experiment is shown in Fig. 4. Exposure of PEC to LPS generated a marked increase in activity (1) during the first 48 h, which decreased by 72 h (data not shown). The dose of LPS was critical (not shown in Fig. 4). In all experiments stimulation required 50 $\mu$g of LPS per ml of culture and was not seen with 20 $\mu$g or less. Incubation of spleen lymphocytes with LPS did not result in fluids with mitogenic activity.

*Lymphostimulatory Activities in Cultures of Activated Macrophages.* PEC from mice injected with various materials known to generate morphologically activated macrophages were tested for the secretion of lymphostimulatory molecules. PEC were harvested from mice injected 3 days previously with LPS i.p. The macrophages exhibited clear morphological signs of activation but, as shown in Fig. 4, were not actively secreting the mitogenic principal. Furthermore, in several other experiments such in vivo LPS-stimulated macrophages responded very weakly, if at all, to challenge with latex beads, despite extensive phagocytosis. Thus, direct addition of LPS to peptone macrophages in vitro (data of Fig. 4) or to normal PEC (data not shown) did stimulate increased secretion. However, it appeared that once a macrophage reached a state of "activation" by LPS, it became refractory to secretion of the lymphostimulatory activities.

Thioglycollate-stimulated PEC had very little basal activity. Furthermore, in three experiments, these macrophages did not increase their production after phagocytosis of latex beads.

In contrast to the results with LPS or thioglycollate-activated PEC, striking effects were seen in cultures of PEC from *Listeria*-infected mice (Table II). In this culture note that the response of thymocytes to a 25% vol/vol concentration of culture fluids was of 75,409 cpm compared to a background of 583 cpm. The results of this experiment are not unique, in six different culture fluids the

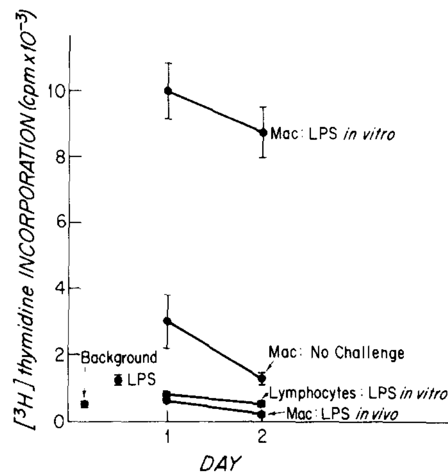


FIG. 4. The same setup as of Fig. 3 also testing the fluids at 25% concentration on thymocyte DNA synthesis. Mac: LPS in vitro, fluids from PEC exposed in culture to LPS; Mac: No challenge, cultures from unstimulated PEC; Lymphocytes: LPS in vitro, fluids from lymphocytes cultured in LPS; Mac: LPS in vivo, fluids from PEC obtained from mice injected with LPS in vivo.

TABLE II
*Mitogenic Activity of PEC from Listeria-Infected Mice*

| Time of culture | cpm incorporation | | | |
|---|---|---|---|---|
| | 5 | 10 | 25 | 50 |
| | *% PEC fluids* | | | |
| Day 1 | 15,440 ± 1,350 | 39,117 ± 1,256 | 75,409 ± 3,653 | 92,203 ± 1,644 |
| Day 2 | 2,294 ± 179 | 5,219 ± 443 | 9,062 ± 1,340 | 11,313 ± 809 |
| Day 3 | 1,671 ± 17 | 2,736 ± 100 | 5,851 ± 780 | 6,513 ± 147 |
| Day 4 | 1,259 ± 101 | 2,453 ± 226 | 4,282 ± 136 | 6,074 ± 493 |

Background cpm was 583 ± 103. Each result is the average of three dishes. The PEC culture fluids were obtained every 24 h for 4 days (first vertical column) and assayed at different concentrations on thymocytes.

range of stimulation at 25% vol/vol concentration ranged from about 20,000 to 75,000 cpm. At the time of harvest, the exudates were sterile and made up of highly activated macrophages.

*The Effects of Activated T Cells on Macrophage Cultures.* The high activity found in PEC from *Listeria*-infected mice contrasted with the meager activity found in PEC activated by LPS or thioglycollate. One possible explanation was the presence of some activated T cells in *Listeria* PEC which could contribute and/or influence the secretion of active molecules. This explanation was found to be true. Figs. 5 and 6 show two key representative experiments. In the experiment shown in Fig. 5 PEC from *Listeria*-infected or normal mice were cultured for 1 or 2 days. The PEC from *Listeria*-infected mice generated highly active mitogenic factor, but the activity decayed during the 24- to 48-h period of culture (Day 2). At the end of 24 h, when the cultures were no longer actively secreting the principles, we added to one set of dishes (or to dishes containing the nonstimulated PEC) a small amount, $3 \times 10^6$, of T lymphocytes from the

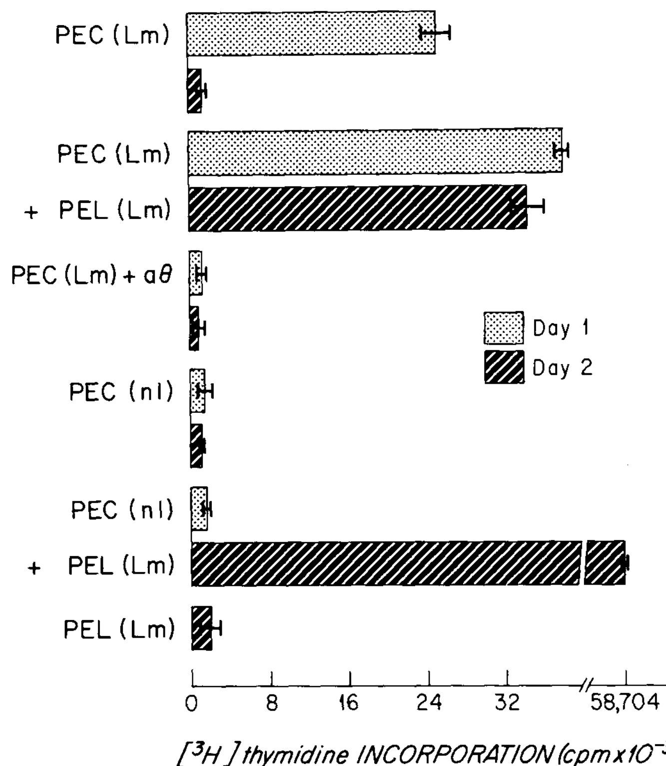


FIG. 5. PEC from *Listeria*-infected (PEC Lm) or normal (PEC Nl) mice were cultured for 1 and 2 days. At the 2nd day peritoneal exudate lymphocytes from *Listeria*-infected mice (PEL Lm) were added to some of the cultures. PEC (Lm) and anti-$\theta$ refers to cultures treated with anti-$\theta$ and complement. All cultures were tested at 25% concentration on thymocytes.

peritoneal cavity (PEL) of the infected mice. This resulted in the secretion of strikingly high amounts of mitogenic factor. By themselves cultures of T lymphocytes (in the presence or absence of $10^{-4}$ M mercaptoethanol) did not generate active principle. Another point to note in the experiment of Fig. 5 is that treatment of the PEC from *Listeria*-infected mice with anti-$\theta$ and complement abolished the high production of mitogenic factor seen during the first 24 h of cultures.

The experiment of Fig. 6 extends the previous results, showing that the addition of activated lymphocytes from *Listeria* exudates, but not from normal exudates, to 48-h culture of *Listeria* PEC resulted again in an increased secretion of the mitogenic principle. Also note that the PEC challenged at 48 h with antibody-coated red cells responded very well, secreting large amounts of material.

Cytological examinations of cultures from normal or *Listeria*-activated PEC to which activated T cells were added showed lymphocytes in between the macrophages and frequent ones attached to them. The number of lymphocytes with ameboid features was high, implying active motility of the activated T cells.

One point concerning the lymphostimulatory activities of PEC from *Listeria*-

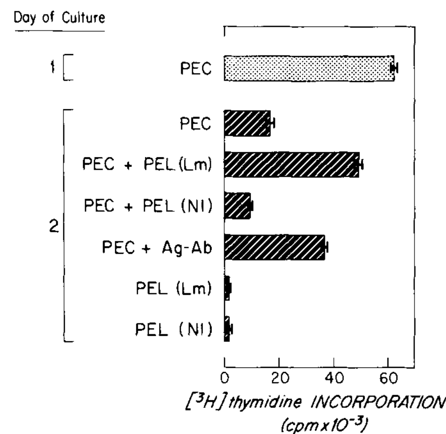


FIG. 6. The experiment has the same basic protocol of Fig. 5 using PEC from *Listeria*-infected mice. At Day 2 the PEC from *Listeria*-infected mice received peritoneal lymphocytes from *Listeria*-infected (PEL Lm) or normal (PEL Nl) mice or antibody-coated sheep red cells (Ag-Ab). Cultures of lymphocytes by themselves are included. All culture fluids were tested on thymocytes at 25% concentration.

infected mice is worth emphasizing. In most experiments with PEC, normal or stimulated, the mitogenic activity and the B-cell differentiating activities have usually run in parallel, but notable exceptions have occurred. We tested five culture fluids from PEC of *Listeria*-infected mice in both assays (i.e., thymocyte DNA synthesis and antibody formation in vitro). All had strong mitogenic activity. In two culture fluids the B-cell activity was negligible; in another the B-cell activity was maintained high during the 24–48-h period of culture, while the mitogenic factor markedly decreased; in the remaining two, both activities ran parallel.

The relationship between lymphocytes and macrophages in the production of the lymphostimulatory activities was studied using antigen-primed T cells. Mice immunized a month previously with F.KLH were boosted with 50 $\mu$g in complete Freund's adjuvant i.p., and a week later the T cells were isolated from the spleen. The T cells were added to cultures of PEC and the culture fluids harvested after 24 h of culture and tested for thymocyte activity. Table III shows the results of one such experiment. Cultures of T cells by themselves did not generate significant activity. The addition of activated T cells to the macrophages increased markedly the production of the factor(s). In experiments not shown, the optimal ratio of T cells to macrophages has been in the order to 5–10. The presence of antigen increased the activity of the T cells, although this was not essential in all experiments. In the experiment of Table III (or in those of Figs. 5 and 6), significant activity was found just by addition of the T cells to the macrophages. However, in other experiments using F.KLH, addition of the antigen was necessary. Note in Table III that cultures of normal lymphocytes, macrophages, and antigen also contained some activity. The requirement for antigen may be a reflection of the state of activation of the T cell.

In one further experiment, we explored whether the F.KLH-activated T lymphocytes interacted best with the normal PEC or PEC activated by thiogly-

TABLE III
*Effect of Immune T Lymphocytes*

| Composition of cultures | | | Incorporation of [$^3$H]thymidine | |
|---|---|---|---|---|
| PEC | Lymphocytes | F.KLH | No PHA | PHA |
| + | – | – | 1,734 ± 187 | 7,534 ± 972 |
| + | Immune: 4 × $10^6$ | – | 9,701 ± 2,106 | 20,746 ± 2,671 |
| + | Immune: 4 × $10^6$ | + | 15,717 ± 605 | 29,321 ± 3,123 |
| – | Immune: 4 × $10^6$ | – | 681 ± 57 | 1,645 ± 21 |
| – | Immune: 4 × $10^6$ | + | 936 ± 47 | 4,053 ± 774 |
| + | Immune: 8 × $10^6$ | – | 10,029 ± 1,087 | 23,959 ± 1,914 |
| + | Immune: 8 × $10^6$ | + | 31,098 ± 2,004 | 37,339 ± 2,328 |
| – | Immune: 8 × $10^6$ | – | 516 ± 165 | 1,532 ± 114 |
| – | Immune: 8 × $10^6$ | + | 498 ± 91 | 1,922 ± 110 |
| + | Normal: 4 × $10^6$ | – | 3,705 ± 282 | 11,224 ± 8,647 |
| + | Normal: 4 × $10^6$ | + | 5,455 ± 880 | 16,470 ± 115 |

The incorporation of [$^3$H]thymidine by thymocytes cultured in the absence or presence of PHA was 608 ± 60 and 1,567 ± 79 cpm, respectively. Not included in this experiment are controls of cultures of normal lymphocytes; these have never generated active materials in subsequent experiments. The culture fluids were tested at 25% concentration.

collate, peptone, LPS, or *Listeria*-infected mice. The PEC from *Listeria*-infected mice were tested after 48 h of culture, by which time there was no secretion of the mitogenic principle. Along the lines seen before, a comparable response was obtained from PEC of normal, peptone, or *Listeria*-infected mice, but little from PEC after injection of thioglycollate or LPS.

## Discussion

The present series of experiments outlined some of the conditions for secretion of the lymphocyte stimulatory factor(s) by macrophages. In our previous study we had shown, in agreement with others, that culture fluids rich in macrophages exerted quite powerful action on lymphocytes in culture. The culture fluids contained a mitogenic principle for thymocytes and to lesser extent for B and T lymphocytes. Besides the thymocyte mitogenic principle, macrophage culture fluids, under appropriate conditions, increased the helper activity of primed T lymphocytes and differentiated primed B cells to active antibody-secreting cells. Whether or not several molecules were responsible for these different effects is not clear to us at present. The fact that on occasion the mitogenic and B-cell differentiating activities were not found in the same culture fluid suggests that there may indeed be at least two different molecules. In our preliminary biochemical studies, both activities were found on a single fraction of about 20,000 daltons (3). However, other subsequent examinations have shown heterogeneity in size of the mitogenic principle, suggesting various molecules or a tendency for a single molecule to polymerize or dissociate; in some instances some dissociation of both activities was noted.

In this study we outlined two conditions that led to increased secretion of the

active products. One was the challenge of macrophages by various agents. Clearly, latex beads, endotoxin, sheep cell-antibody complexes, *Listeria* organisms, even Be salt produced an increased secretion of the activities. The increased activity was seen during 1 or 2 days of culture and then declined. The increased secretion was consistent but with some degree of variation from experiment to experiment. This phenomenon is consistent with several observations made on secretion of enzymes and other molecules by macrophages. Plasminogen activator-like molecules (13), elastase (14), collagenase (15), complement factors (16), pyrogen (17), and some lysosomal enzymes (18, 19) are secreted best upon phagocytic challenge. However, our observations differ from some of the published reports in two important aspects: *(a)* while an undigestible particle like latex stimulated some enzyme secretion continuously (13–15), the secretion of the lymphostimulatory activities was usually short lived; *(b)* while phagocytic stimuli best worked on "primed" or activated macrophages (20), this was not the case with the secretion of the activities described herein; it was clear from the experiments with LPS and thioglycollate that no direct relationship was found between morphological appearance and the secretion of lymphostimulatory material. The behavior of these PEC contrasted with *Listeria*-activated PEC, which were capable of responding to external stimuli but not as well as nonstimulated macrophages (Fig. 5).

A second condition for the secretion of the lymphostimulatory molecules was the presence of activated T cells in the culture together with the macrophages. Clearly, in all instances, the activated T-cell-macrophage culture did not necessitate the addition of antigen to secrete the factor (experiments of Figs. 5 and 6 and Table III), although antigen challenge increased the production (Table III). At this point, one ought to question what cell in the T-cell-macrophage mixtures secretes the factor – the macrophage, the activated T cell, or both. We know from experiments reported previously (3) that macrophages secrete the activities under basal conditions. Furthermore, the secretion as shown herein increases following phagocytosis. In contrast, cultures of activated T cells do not contain the active principle. We are inclined to ascribe the increased secretion in the activated T-cell-macrophage mixture to the macrophage affected in whatever form, by the activated T cell. However, it would not surprise us if the converse also is true and that the T cells upon interaction with the macrophage could be made to secrete the activities. Further experiments are in progress attempting to dissect this phenomenon.

We consider it likely that secretion of the active lymphostimulatory moieties may play some role in immune induction. We envision three conditions where this could occur: *(a)* after the uptake of a large bolus of antigen by macrophages of lymphoid tissue; *(b)* subsequent to interaction of the phagocytes with adjuvants (such as LPS, Be); or *(c)* after the generation of highly activated T lymphocytes. These conditions, however, are restricted by the "activity" of the phagocytes inasmuch as highly stimulated cells appear to become refractory. The observation that the activity or maturation state of the macrophage conditions the extent of secretion of some molecules but not others indicates a rather sophisticated regulation of its secretory process which may explain the protean role of macrophages in immunity and in inflammation.

## Summary

Cultures of peritoneal exudate cells rich in macrophages were studied for the secretion of lymphostimulatory molecules. Two conditions produced increased secretion: *(a)* addition to the cultures of various agents that readily interacted with macrophages, such as latex particles, antibody-coated red cells, endotoxin, *Listeria* organisms, or Be salt; or *(b)* addition of activated lymphocytes.

In the first case the increased activity was found during the first 24 or 48 h after uptake of the stimuli. Increased activity was found in normal or peptone-stimulated macrophages but not in macrophages after injection of endotoxin or thioglycollate.

The addition of T lymphocytes from *Listeria*-infected mice to macrophage cultures increased greatly the activities. This increase was also produced by addition of antigen-primed T cells together with antigen. The lymphocytes by themselves did not secrete active factors.

The lymphostimulatory activities were tested on thymocyte DNA synthesis and on antibody formation in vitro. The latter assay was done on spleen cells from immunized mice where one striking effect was the stimulation of differentiation to antibody-secreting cells. Some dissociation of both activities (thymocyte DNA synthesis and B-cell differentiation) was observed with selected culture fluids.

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