THE PRODUCTION OF MIGRATION INHIBITION FACTOR BY B AND T CELLS OF THE GUINEA PIG*

BY TAKESHI YOSHIDA, HIDEKICHI SONOZAKI, AND STANLEY COHEN

(From the Department of Pathology and The Center for Immunology, State University of New York at Buffalo, Buffalo, New York 14214)

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It is now well known that sensitized lymphocytes release various effector substances when stimulated by specific antigen in vitro (1). These substances have been collectively defined as "lymphokines." The elaboration of some, if not all of these, is correlated with the state of delayed hypersensitivity of the lymphocytes donor (1-2). Although a variety of observations have provided indirect evidence that the various manifestations of cellular immunity are mediated by the T cell subpopulation of lymphocytes, there are only very few observations which show directly that any of the various lymphokines are produced by T cells (3). This has been mainly because of the lack of a suitable marker for T cells in guinea pigs, in which species most of the studies on delayed hypersensitivity have been performed.

Recently it has been shown that lymphocytes of various species including the guinea pig may be divided into two populations on the basis of whether or not they possess receptors for the third component of complement on their surface (4). These populations, known as complement receptor lymphocytes (CRL)1 and noncomplement receptor lymphocytes (NCRL), have been shown to correspond respectively to B cells and T cells (5, 6).

Previously, we have reported that the macrophage disappearance reaction (MDR) is mediated by NCRL through the release of a soluble factor from such cells which affects the behavior of macrophages in peritoneal exudates in the intact animal (3). CRL had no detectable activity in this regard. These observations therefore provided evidence for the production of at least one lymphokine by a subpopulation of lymphocytes corresponding to T cells.

The present series of experiments were designed to study the ability of puri-
Materials and Methods

Immunization.—Female Hartley guinea pigs weighing 350–400 g were immunized with footpad injections of 100 μg of egg albumin (EA), dinitrophenylated egg albumin (DNP-EA), or dinitrophenylated bovine serum albumin (DNP-BSA) in complete Freund adjuvant (Difco Laboratories, Detroit, Mich.), or the adjuvant alone. In a preliminary experiment, a group of 40 nonimmune animals were skin tested with 20 μg of tuberculin purified protein derivative (PPD) (Parke, Davis, and Co. Detroit, Mich.) to demonstrate that those guinea pigs were not sensitive to this antigen. All these animals were negative to skin test. This was done in an attempt to exclude the possibility of pre-existing sensitization to PPD in our animal population. However, all the guinea pigs which served as normal controls, as a source of “nonimmune” lymphocytes, in the present experiments were not pretested, since such treatment is known to either sensitize or to enhance a pre-existing subthreshold sensitivity (7). However, they were obtained in the same shipment and from the same supplier as those found to be PPD negative.

Lymphocyte Suspensions.—Sensitized lymphocytes were obtained from lymph nodes draining the sites of antigen injection in guinea pigs, and were removed 8 or 9 days after immunization. Normal lymphocytes were obtained from axillary, popliteal, inguinal, and iliac lymph nodes, or from spleens of normal guinea pigs. Cell suspensions were made in RPMI 1640 medium (Associated Biomedic Systems, Inc., Buffalo, N.Y.), supplemented with penicillin (50 U/ml) and streptomycin (50 μg/ml).

Preparation of Indicator Erythrocytes.—Sheep erythrocytes were sensitized with antibody and guinea pig complement as described previously (3). Briefly, equal volume of a 5% suspension of erythrocytes and a 1:200 dilution of rabbit antimouse hemolysin (amboceptor, Hyland Laboratories, Los Angeles, Calif.) in Veronal-buffered saline (VBS), pH 7.4, were incubated at 37°C for 30 min. The sensitized cells were washed twice and resuspended to the original concentration. An equal volume of a 1:100 dilution of fresh guinea pig serum (as a complement source) in VBS was added and incubated at 37°C for 20 min. After three washings in VBS and two washings in phosphate-buffered saline (PBS), pH 7.4, containing 0.01 M EDTA, a final suspension of sensitized erythrocytes (EAC) were adjusted to 1 × 10⁹/ml. These cells, which do not lyse spontaneously, were used for the identification and separation of CRL.

Separation of CRL and NCRL.—The cell suspensions prepared as described above were resuspended in EDTA-PBS. Equal volumes of EAC (1 × 10⁹/ml) and cell suspension (5 × 10⁷/ml) were mixed and incubated at 37°C for 15 min to produce erythrocyte rosettes around the CRL present in the suspension. Then an equal volume of a 1:50 dilution of amboceptor was added, and the mixture incubated for another 20 min. This caused agglutination of the rosette-bearing lymphocytes. The preparation was then centrifuged at 1,500 rpm for 4 min and resuspended in the same buffer. The suspension was incubated at 37°C for 30–40 min to allow clumping and settling to occur. The suspension was left standing at 4°C for 20 min and the rosette-free supernatant which contained NCRL were removed. Then more buffer was added to resuspend the rosette-containing cells. These procedures were repeated four to five times to obtain the final CRL-rich cell suspension. The removal of the rosette erythrocytes on the CRL was achieved by treatment with C3 inactivator as described previously (3). In some experiments this was accomplished by treatment with 0.5% NH₄Cl solution as reported elsewhere (8). These procedures resulted in a final population of lymphocytes containing 85–90% of CRL. The recovery ratio of CRL was 20–30%. NCRL had a purity of greater than 95% and their recovery ratio was 30 to 50%.

MIF Production.—The lymphocytes were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air at a density of 1 × 10⁷ or 5 × 10⁶/ml except where otherwise noted. Usually no serum was included in the culture medium. However, 5% normal guinea pig serum was
added when CRL or NCRL were cultured separately since the viability of these cells and the resulting MIF production was higher in such medium. After 24 h of incubation, the supernatants were collected by two centrifugations at 3,000 rpm for 30 min, and then assayed for MIF activity without concentration. In those studies involving Sephadex gel filtration, the supernatants were concentrated by lyophilization (see below). Appropriate amounts of antigen or other test substance were added to reconstitute the control supernatants which had been cultured in the absence of these substances. In one set of experiments, endotoxin lipopolysaccharide was used to induce MIF. Two preparations were used: *Escherichia coli* 055: B5 lipopolysaccharide (Difco) and *Serratia marcescens* endotoxin (Difco). These gave identical results, which are described below.

**Migration Inhibition Test.**—This assay was performed as described previously (9, 10). The migration index was calculated as reported there; in addition, the percent inhibition was calculated as follows:

\[
\% \text{Inhibition} = 100 - \text{Migration Index}.
\]

**Macrophage Disappearance Reaction (MDR).**—The MDR was performed as described previously (11, 12). Briefly, nonimmunized guinea pigs bearing 4-day glycogen-induced peritoneal exudates received intraperitoneal injections of lymphocytes with or without specific antigen. Peritoneal exudates were collected 5 h later. Total cell counts of the suspensions as well as differential counts on Giemsa-stained smears were performed. The percentage of macrophage loss was calculated from the difference between the average count for that cell type in the exudates of the control animals and the animals which had received antigen as well as donor cells.

**Sephadex Gel Filtration.**—Supernatants obtained from the culture of normal CRL with PPD were pooled. 50 ml of such material was concentrated to 3 ml by lyophilization. After removing insoluble debris by centrifugation at 3,000 rpm for 30 min, the preparation was applied on a 2.5 × 50 cm Sephadex G-100 column, which was equilibrated and eluted with PBS, pH 7.4. The flow rate was adjusted to 7 ml/h and 3 ml fractions were collected. Six pooled effluent fractions were collected and concentrated 10-fold as described previously (13, 14). Control supernatants were treated exactly the same way. All of these fractions, in 4 ml of final volume, were dialyzed against RPMI 1640 medium. After sterilization by micropore filtration the various samples were assayed for MIF activity.

### RESULTS

**Production of MIF by NCRL and CRL.**—Groups of guinea pigs were immunized with egg albumin, DNP-EA or DNP-BSA in complete Freund’s adjuvant, or with the adjuvant only. CRL and NCRL were isolated from regional lymph nodes as described. In this set of experiments, the CRL-rich suspensions had purity of greater than 90% and the NCRL, greater than 95%. Each population of cells was cultured in the presence or absence of its own specific antigen at the cell concentration of 6 × 10⁶/ml for 24 h. Viable cell recoveries ranged from 57 to 65% (by Trypan blue exclusion studies) with no significant difference between control (antigen-free) and experimental (antigen-containing) cultures. These figures were obtained for PPD as well as the other test substances. Similarly no significant differences in viability were found between CRL and NCRL cultures. These viability figures are somewhat lower than those obtained when using unfractionated lymphocytes (70–80%); this is unavoidable in spite of the mild conditions for separation. The MIF activity of
such supernatants was tested using normal guinea pig peritoneal macrophages as indicator cells. The results are shown in Table I. Each of the antigens used could induce MIF production when added to cultures of NCRL from animals sensitized to that antigen. There was no significant difference with respect to EA, DNP-EA, or DNP-BSA. The CRL-rich suspensions could not make detectable MIF when cultured with the appropriate antigen from this group. Since all animals were immunized with antigens in complete Freund’s adjuvant, all were sensitized to PPD. Interestingly, unlike the other antigens used, PPD could stimulate the CRL-rich population as well as the NCRL-rich population to produce MIF in culture. As mentioned above, the CRL-rich suspensions still contained approximately 10% NCRL. It is possible that PPD is a sufficiently “good” antigen to activate this small number of T cells, although the other antigens were not. This is not likely since the total amount of MIF activity recovered from the CRL suspensions was identical to that obtained from the NCRL suspensions. Nevertheless, this possibility was investigated by studying the dose-response characteristics of MIF production in this system.

**MIF Production as a Function of NCRL Number.**—In the method we have used to separate the two subpopulations of lymphocytes, one can achieve greater purity of NCRL than CRL. Nevertheless, as stated above, each population retains some degree of contamination by the other. We therefore felt it of importance to compare the relative abilities of different concentrations of NCRL to produce MIF under the culture conditions used, when PPD was used as the stimulating agent. NCRL and CRL were separated from lymphocyte suspensions obtained from lymph nodes of guinea pigs immunized with complete Freund’s adjuvant but no other antigen. As shown in Table II, suspensions of unfractionated lymphocytes (“whole” suspensions) or of NCRL could release MIF into culture at a cell concentration of $10 \times 10^6$/ml. At this cell concentration, NCRL could produce MIF at a level identical to that of the whole suspension. Reducing the concentration of NCRL reduced the MIF activity recoverable in the supernatants. $5 \times 10^6$ NCRL/ml led to 27.0% migration inhibition, and when the dose was reduced further to $1 \times 10^6$/ml,

| Antigen * | CRL | NCRL |
|-----------|-----|------|
| EA        | $-2.6 \pm 7.2$ § | $30.0 \pm 2.4$ |
| DNP-EA    | $-6.4 \pm 4.2$ | $28.1 \pm 3.0$ |
| DNP-BSA   | $7.6 \pm 8.4$ | $42.0 \pm 5.6$ |
| PPD       | $35.1 \pm 5.9$ | $30.9 \pm 8.4$ |

* Antigen in culture; 50 µg/ml.
‡ Cell concentration; $6 \times 10^5$/ml.
§ % Inhibition = (1 – area exp/area cont) × 100.

![Image](image.png)
no detectable MIF activity was recoverable at all. The CRL-rich suspensions which were shown in Table I to give rise to supernatants with 37.6% migration inhibition contained less than 0.6 × 10^6 NCRL/ml. Thus, contamination of that preparation by NCRL cannot be the explanation of its activity.

Stimulation of Nonimmune CRL by PPD.—In the experiments described above, CRL and NCRL from tuberculin-sensitized guinea pigs appear equally capable of producing MIF when stimulated by PPD in culture. Because of the discrepancy between the results with PPD and those with the other antigens used, we explored the possibility that the activation of CRL by PPD might be "nonimmunologic" in nature. Accordingly, CRL and NCRL suspensions were prepared from lymphocytes obtained from lymph nodes on spleens of normal guinea pigs. These guinea pigs were not pretested with PPD, but, as indicated above, a random sampling of other animals from the same supplier showed them all to be negative to skin test with PPD. Such normal CRL and NCRL were incubated with EA, DNP-EA, or PPD for 24 h. The culture supernatants were checked for MIF activity. Percent migration inhibition was calculated in comparison with the migration in control supernatants which were cultured without any stimulating substances but reconstituted with these after the incubation. As expected, neither EA nor DNP-EA could stimulate either the normal CRL or NCRL to produce MIF. However, tuberculin PPD could stimulate normal CRL from either lymph node or spleen, but not normal NCRL from these sources, to produce MIF. 50 μg/ml of PPD resulted in preparations with approximately 30% migration inhibition. These results are summarized in Table III. Not shown are data for 10 μg/ml PPD (21% inhibition) and 1 μg/ml PPD (14% inhibition). This latter figure does not represent significant activity by the usual criteria for MIF studies, but is significantly higher than the values obtained with NCRL at any dose of PPD (<6%).

Characterization of CRL-Derived MIF.—The macrophage migration inhibitory substance obtained from the stimulation of nonimmune CRL by PPD need not be identical to the "classic" MIF obtained by the specific stimulation of sensitized T cells. In order to characterize this material, column chroma-
tography studies, on Sephadex G-100, were performed on the active supernatants from the PPD-stimulated nonimmune CRL. Six approximately equal volumes of eluate fractions were pooled and tested for MIF activity. Inhibitory activity of each fraction was compared with that of corresponding fractions obtained by the gel filtration of control supernatants. MIF activity was mainly found in Fraction III whose molecular weight corresponded approximately to that of ovalbumin, and also, in small amount, in Fraction V whose molecular weight corresponds approximately to that of cytochrome C. These results are shown in Fig. 1. The elution pattern of this CRL-derived MIF is quite similar to that obtained by column chromatography on Sephadex of culture supernatants obtained from antigen-specific stimulation of sensitized lymphocytes, as reported by us and others (13-15).

### TABLE III

| Lymphocyte source | Antigen* | CRL | NCRL |
|-------------------|----------|-----|------|
| Lymph node        | EA       | 4.6 ± 8.8§ | 1.0 ± 2.9 |
|                   | PPD      | 31.2 ± 5.3  | 5.8 ± 6.3  |
| Spleen            | EA       | 9.2 ± 5.1   | 9.0 ± 3.8  |
|                   | DNPEA    | 8.8 ± 4.0   | 9.3 ± 4.0  |
|                   | PPD      | 31.8 ± 4.0  | 1.5 ± 2.5  |

* Antigen concentration: 50 μg/ml.
† Cell concentration: 10 × 10⁶/ml.
§ % Inhibition.

Fig. 1. Sephadex G-100 gel filtration of supernatant obtained from normal CRL cultured with PPD. Arrows indicate the positions where marker proteins eluate; guinea pig albumin (mol wt 67,000) ovalbumin (mol wt 45,000) and cytochrome C (mol wt 12,400). Fractions of 3 ml/tube were collected and pooled into six approximately equal volume (30 ml) fractions. Each fraction after 10-fold concentration was tested for MIF activity.
In addition, other physicochemical characteristics of this MIF preparation were studied. The material was not cytotoxic for macrophages on the basis of Trypan blue exclusion studies. It did not lose its MIF activity by heating at 56°C for 30 min, but lost activity by heating at 80°C for 1 h. Trypsin digestion of the material at the enzymatic concentration of 5 mg/ml resulted in a total loss of MIF activity. These characteristics were also identical to that of classical MIF.

**Stimulation of CRL by Endotoxin Lipopolysaccharide.**—Since PPD could stimulate CRL (B cells) to produce MIF, it was of interest to determine whether or not endotoxin lipopolysaccharide, which is a known B cell mitogen in the guinea pig (16), could do so as well. Accordingly, 20 μg/ml of endotoxin lipopolysaccharide (Difco) was added to the cultures of normal CRL or NCRL. It was found that supernatants obtained from CRL cultured in the presence of this material showed macrophage migration inhibitory activity, although the activity recovered was somewhat weaker than that obtained by stimulation with 20 μg/ml of PPD, averaging 24% inhibition.

**Macrophage Disappearance Reaction (MDR).**—As stated previously, the MDR elicited by pure soluble protein antigen has been found to be mediated by T cells. Because of the observations reported here, those studies were repeated using PPD as eliciting agent. In this experiment, CRL and NCRL were separated from lymph nodes of guinea pigs immunized with egg albumin in complete Freund's adjuvant. The results are shown in Table IV. 2 × 10⁸ cells of NCRL and 40 μg of egg albumin when injected into nonimmunized guinea pigs bearing 4-day glycogen-induced peritoneal exudates could induce the MDR with a 77.5% loss of peritoneal macrophages, but the same amount of CRL and antigen was entirely negative, confirming the previously reported results (3). However, PPD could stimulate both CRL and NCRL, causing 50.5 and 69.3% loss of macrophages, respectively. These figures are the average of three independent experiments each performed in triplicate. Thus, the in vitro results reported above are confirmable in an in vivo system. The ability to transfer the MDR with nonimmune lymphocytes and PPD was not examined in this set of studies.

| Lymphocytes | Antigen* | Macrophage loss % |
|-------------|----------|-------------------|
| NCRL†       | EA       | 77.5              |
|             | PPD      | 69.3              |
| CRL‡        | EA       | 3.8               |
|             | PPD      | 50.5              |

* Cell dose, 2 × 10⁶; purity 95%.
† Cell dose, 2 × 10⁶; purity 88%.
‡ Antigen dose 40 μg.
DISCUSSION

In the present study, we have separated guinea pig lymphocytes into two populations on the basis of the presence or absence of a receptor for the third component of complement on their cell surfaces. It has been well documented that the cells which lack this receptor (NCRL) correspond to T cells, and that the cells which possess this receptor (CRL) correspond to B cells (4–6). Making use of these associations, we have shown that several antigens are capable of stimulating relatively pure populations of T, but not B cells, from appropriately immunized animals, to produce and/or release MIF into culture supernatant fluids in vitro. This is analogous to the results of our previous studies on the macrophage disappearance reaction (3) which represents an in vivo analog of the migration inhibition reaction. T cells, but not B cells were shown to be capable of mediating the macrophage disappearance reaction, when transfused with specific antigen into nonimmune hosts (3). This reaction could be accomplished even when the cells were enclosed in micropore chambers. Those experiments, and the studies reported here, represent the first direct documentation that lymphokines are produced by T cells. These results are not surprising in the light of the overwhelming evidence that the various manifestations of cell-mediated immunity are dependent upon T cell function (17, 18). What is surprising is that PPD, of the several antigens tested, was capable of inducing similar activities in a population composed predominantly of B cells. B cells activated by PPD could mediate both the migration inhibition reaction and the macrophage disappearance reaction. The substance responsible for migration inhibition had physicochemical properties consistent with those of "classic" MIF and may well be identical to it. Quantitative considerations, as presented in the previous section, argued against the possibility that the contaminant T cells in our B cell-rich suspensions were the cause of this activity.

A finding of major importance in this study was that PPD could also stimulate B cell-rich suspensions obtained from nonimmunized donors to produce MIF. Unlike the situation when sensitized cells were used, PPD could not stimulate the T cells from the nonimmunized donors. This fact lends further support to our contention that this group of animals was not presensitized to products of the tubercle bacillus. Moreover, it shows that the ability of the B cell suspensions to produce MIF could not be due to the presence of the small numbers of T cells in those B cell suspensions. Since similar levels of activity were obtained when PPD was added to the CRL from sensitized donors, these findings reinforce our conclusion, based upon the quantitative considerations above, that the results were not due to contaminant T cells in that situation as well. The possibility that the B cells are interacting with, and enhancing the ability of a small number of critical T cells in this system to make MIF is unlikely, not only because it would represent a lymphocyte-lymphocyte interaction opposite to the well-known interaction which leads to the enhance-
ment of B cell function by T cells (helper function), but also because it would represent the consequence of such inverse interaction in a nonimmune as well as immune system. In any case, even if this alternate possibility should turn out to be correct, the observations reported here point to a previously unreported capability of B cells.

It is of interest that the phenomenon reported here has never been observed in controls for the usual indirect migration inhibition reaction performed with PPD as antigen. In the typical experimental setting, the control involves culturing an aliquot of sensitized lymphocytes without antigen, and then reconstituting the supernatant fluids with antigen at the end of the incubation period. This supernatant, containing PPD, but no MIF, is added to peritoneal exudates from normal animals. In the light of the observations reported here, the PPD might be expected to activate the B cells in that exudate. The failure to detect such a reaction is most likely related to our previously reported observations on the very low content of B cells in peritoneal exudates used in migration inhibition studies. In those preparations, more than 95% of the lymphocytes are T cells (3). Parenthetically, this fact also provides a partial explanation for the relatively greater ability of peritoneal exudate cells, as compared to lymph node suspensions, to mediate various reactions of cellular immunity (19-21).

It is well-known that various mitogens can stimulate normal lymphocytes from nonimmune animals to produce MIF-like substances (22, 23). In at least one such study, it has been shown that the material produced has characteristics which are identical to MIF produced by antigen-stimulated sensitized lymphocytes (22). Recently, Sultzer and Nilsson have reported that PPD is also mitogenic, but that unlike most of the known mitogens, it appears to act selectively on B cells of the mouse (24). To confirm this point, we have studied thymidine incorporation in normal guinea pig CRL and NCRL during PPD stimulation. PPD (50 µg/ml) produces an approximately threefold increase in thymidine incorporation in CRL but has no effect on NCRL. These results, though significant, are slightly less than those reported by Sultzer and Nilsson who obtained incorporation ratios of 3.8 to 4.5 using cells from nude mice. Since PPD could activate lymphocytes from nonimmune, as well as immune donors in these studies, it appears as if its MIF-inducing ability might well be related to its mitogenic ability. It is, of course, possible that the active substance in our studies and in the work of Sultzer and Nilsson was not PPD itself but some contaminant within the preparations used. The fact that we could obtain MIF production by incubating B cells with endotoxin lipopolysaccharide, another mitogenic substance which affects B cells (16), lends credence to this possibility, and in addition, provides support for the notion that MIF production by nonimmunized B cells could be related to a mitogenic effect of the stimulating substances.

There are several speculations one may make about the biologic significance
of these observations. First, our data provide no evidence that B cells from immune animals are more effective in producing MIF than those from non-immune animals. Thus, the induction of MIF by stimulation of sensitized lymphocytes with specific antigen probably represents a purely T cell phenomenon. This is analogous to the situation in which the in vitro stimulation of DNA synthesis in lymphoid cells from immunized animals by specific antigen was studied. Here, removal of T cells by lysis with an appropriate antiserum largely abolished the capacity of the cell population to manifest this response (25), whereas enrichment of T cells by glass bead column chromatography enhanced the response (26). Thus, in two distinct in vitro systems, both of which correlate with cell-mediated immunity, specific antigen activates T rather than B cells. The situation with respect to nonantigenic activation is quite different. Nonspecific agents may function as B cell mitogens, inducing blast transformation and DNA synthesis. These include not only the substances studied in the present experiments, but also agents previously considered to be only T cell mitogens, such as pokeweed mitogen, phytohemagglutinin, and concanavalin A (16). Similarly, as we have shown, nonimmunologic activation of a B cell population can lead to the appearance of MIF activity. This raises the possibility that various other lymphokines, in addition to MIF, could be generated by similar mechanisms.

In vivo, antigen must somehow lead to a wave of proliferative activity in an appropriate subpopulation of the B cell series, in order to account for the observed kinetics of antibody formation in both the primary and secondary response. As stated above, antigen-induced activation of B cells for proliferation has not been observed in vitro. Since there is abundant evidence that lymphocytes which play a role in cell-mediated immunity may produce lymphokines with mitogenic activity (reviewed in 27), it is possible that at least some of these agents might act selectively on B cells. In other words, T cells might function in vivo in a manner similar to that of endotoxin lipopolysaccharide or PPD in vitro. In support of this possibility, it has been shown that endotoxin lipopolysaccharide could alter thymic-deficient mice so that they could respond to thymic-dependent antigens (28), and that endotoxin could induce an immune response in mice to a hapten coupled to a nonimmunogenic carrier (29). These experiments are difficult to interpret since unlike the situation in guinea pigs endotoxin lipopolysaccharide stimulates T cells as well as B cells in the mouse (16). Nevertheless, an endogenous mitogen for B cells might provide one mechanism by which the “helper” function of T cells expresses itself. It has been shown that “helper” functions may be mediated by soluble factors released by T cells (27, 30). Although a lymphokine specifically mitogenic for B cells has not yet been described, it is of interest that humoral factors produced by thymocytes may potentiate the ability of concanavalin A to activate B cells (31). Moreover, activated lymphocyte cultures have a small but definite mitogenic effect on spleen cells from nude mice, which lack T cells (32). This
possibility is currently under investigation in our laboratory. In any case, once B cell activation occurs, production of lymphokines by these cells, as well as T cells, would provide for further amplification of the immune response.

As a final comment, it has been shown that MIF, or substances with similar biologic and physicochemical properties may be found in certain replicating cultures of nonlymphoid cells (33, 34). Also, we have recently shown that migration inhibition activity (35, 36), as well as certain other lymphokine activities such as macrophage and lymphocyte chemotaxis (37), may appear after the in vitro or in vivo infection of nonlymphoid, as well as lymphoid cells by mumps or Newcastle disease virus. These results, taken in conjunction with those reported here, suggest that lymphokine production, rather than merely representing an effector mechanism for cell-mediated immunity, represents a general biologic phenomenon which may play a role in various aspects of host defense. In this view, the lymphocyte has acquired some specialized means for triggering this production, not available to other cells. B cells may do so by mitogenic activation, and T cells by either mitogenic or antigenic activation.

SUMMARY

Stimulation of sensitized lymphocytes by specific antigen in vitro leads to the production of migration inhibition factor (MIF). In the case of the pure soluble protein, or hapten-protein antigens used in the present studies, this MIF production was a property of the T lymphocytes in the cell suspensions. When PPD was used, B cells, as well as T cells, produced MIF. Similarly, PPD could stimulate B cells to mediate the macrophage disappearance reaction, a reaction which is known to be a T cell-dependent in vivo manifestation of cell-mediated immunity. Suspensions of lymphocytes from nonimmune donors could also be stimulated by PPD; in this case, B cells, but not T cells, produced MIF. The factors produced by the two lymphocyte subpopulations appeared to be similar, if not identical, on the basis of physico-chemical criteria. It is suggested that PPD stimulates B cells for MIF production because of its role as a B cell mitogen. The ability of endotoxin lipopolysaccharide, another B cell mitogen, to also induce MIF production by B cells supports this contention. Thus, although activation of lymphocytes for MIF production by specific antigen is a property of T cells, B cells as well as T cells may be so activated by agents which act nonspecifically. This may prove to have implications for in vivo events involved in immunization. In addition, these observations lend further support to the concept that lymphokine production represents a general biologic phenomenon in addition to playing a role in the effector mechanisms for reactions of cell-mediated immunity.

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