STUDIES ON MEDIATOR PRODUCTION BY HIGHLY PURIFIED HUMAN T AND B LYMPHOCYTES*

By ROSS E. ROCKLIN,‡ RICHARD P. MacDERMOTT, LEONARD CHESS, STUART F. SCHLOSSMAN, and JOHN R. DAVID

(From the Departments of Medicine, Harvard Medical School, the Robert B. Brigham Hospital, and the Sidney Farber Cancer Center, Boston, Massachusetts 02120)

When lymphocytes obtained from persons or animals exhibiting delayed hypersensitivity are stimulated in vitro by specific antigen, they produce a number of soluble substances with various biologic activities including migration inhibitory factor (MIF)[1] and lymphocyte mitogenic factor (LMF) (1–4). Since delayed hypersensitivity reactions have been generally assumed to be mediated by thymus-dependent (T) lymphocytes, it has been further assumed that antigen-induced production of MIF was by T lymphocytes and thus its presence or absence reflected T-cell function. The correlation of macrophage migration inhibition with delayed hypersensitivity and not with antibody production (2, 5, 6), the production of MIF by lymphocytes from patients with sex-linked agammaglobulinemia but not thymic aplasia (7), and the lack of MIF production in numerous immunodeficiency diseases linked with depressed delayed hypersensitivity and normal antibody production (8, 9) are part of the foundation for this conclusion.

The availability of a new method of cell separation utilizing affinity column chromatography which allows the quantitative recovery of virtually pure populations of human T and B lymphocytes has permitted a re-evaluation of the functional properties of T and B cells (10, 11). Using this technique, Chess et al. have recently shown that both T and B human blood lymphocytes respond to mitogens such as phytohemagglutinin (PHA), concanavalin A, and pokeweed, but only T-cell populations proliferate in response to specific soluble or cell surface antigens (10, 11).

In the present study, we have used this affinity column separation technique to critically evaluate the hypothesis that antigen-induced mediator production is the exclusive property of T lymphocytes. Highly purified T- and B-lymphocyte populations were stimulated by specific antigen and their ability to produce two

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[1] *Abbreviations used in this paper:* LMF, lymphocyte mitogenic factor; MIF, migration inhibitory factor; P, preincubated; PHA, phytohemagglutinin; PPD, purified protein derivative of tuberculin; R, reconstituted; SI, stimulation index; SK-SD, streptokinase-streptodornase.

lymphocyte mediators, MIF and LMF, was assessed. Whereas only T cells made LMF, both T and B cells produced MIF (12). Thus, the production of MIF, while correlating with cutaneous delayed hypersensitivity, does not solely reflect T-cell function but B-cell function as well. Under these circumstances, the explanation for the production or lack of production of MIF by lymphocytes obtained from patients with immunodeficiency disorders requires reinterpretation.

Materials and Methods

Cell Separation Technique. The method of Chess et al. was used (10, 11). Purified rabbit anti-human Fab immunoglobulin was coupled to Sephadex G-200 using cyanogen bromide (10). Heparinized blood was drawn by venipuncture from donors exhibiting cutaneous delayed hypersensitivity to purified protein derivative of tuberculin (PPD), *Candida*, or streptokinase-streptodornase (SK-SD) as well from donors lacking these sensitivities (13). Mononuclear cells were separated by centrifugation in a Ficoll-Hypaque gradient (14). 98% of the mononuclear phagocytes were removed by incubation with carbonyl iron and subsequent exposure to a magnetic field. The lymphocyte-rich population, in medium TC-199 containing 5% fetal calf serum and 10^{-4} M sodium EDTA, was passed over a Sephadex anti-Fab column. The effluent cell population (nonretained) contained less than 2% cells bearing surface immunoglobulin when evaluated by immunofluorescent-staining techniques (10). These cell populations will be referred to as T cells. The cells retained in the column were then eluted with medium TC-199 containing 1% normal human immunoglobulin (10). These Ig-eluted cells were 95–98% surface immunoglobulin positive as assessed by immunofluorescence and are referred to as B cells. There was a 95–100% recovery of the starting cell population using this immunoadsorbent technique. Cell viability as determined by trypan blue exclusion was greater than 95%.

Production of Lymphocyte Mediators. The unseparated and column purified lymphocyte populations were washed twice in serum-free medium. They were adjusted to 5×10^6 lymphocytes/ml in medium TC-199 containing 10% gamma globulin-free horse serum in the absence or presence of 10 μg/ml PPD, 50 U/ml SK-SD, or 0.01 ml/ml stock solution of *Candida albicans*, and incubated for 48 h at 37°C in a 5% CO_2-95% air atmosphere. The cultures were centrifuged to remove the cells; supernates obtained from the control culture were reconstituted with antigen in the original amount. In most experiments, these supernates were assayed directly for MIF or LMF activity. In some experiments, the supernates were dialyzed, lyophilized, and applied to Sephadex G-100 columns, and the fractions were concentrated and assayed.

Column Chromatography. Lyophilized control and active supernates from T- or B-cell cultures were reconstituted to 2 ml with phosphate-buffered saline (pH 7.4) and applied to 2.5 × 100-cm columns containing Sephadex G-100 as previously described (15). The columns were calibrated with [^{125}I]RSA and [^{125}I]chymotrypsinogen as markers having mol wt of 68,000 and 23,000 daltons, respectively. The eluate was pooled into four fractions: fractions I and II contained material eluting from the void volume of the column to the beginning of the elution of albumin; fraction III (Kd 0.08–0.19) contained material eluting with albumin; fraction IVb (Kd 0.33–0.45) contained material eluting with molecules having the size of chymotrypsinogen and is the region where peak MIF activity from unseparated lymphocyte populations is found (15); and fraction IVa contained material between the albumin and chymotrypsinogen markers. Each fraction was dialyzed against distilled water and lyophilized. Before assay the fractions were made up to $\frac{1}{10}$ the original volume in medium TC-199 containing 10% horse serum; these were sterilized by passage through a Millipore filter. The paired control and active fractions were assayed for MIF and LMF activity as described below.

Assay for MIF Activity. Unconcentrated supernates or 10-fold concentrated Sephadex G-100 fractions from control or antigen-stimulated T- or B-cell cultures were assayed for MIF activity on guinea pig macrophages in capillary tubes (16). The area of migration was drawn at 24 h, measured by planimetry, and the percent migration inhibition calculated by the following formula: % migration inhibition = 1.0 − (area of migration in "active" supernate)/(area of migration in control supernate) × 100.

Assay for LMF Activity. Active (preincubated [P]) and control (reconstituted [R]) supernates

were assayed for mitogenic activity using lymphocytes from normal donors who lacked sensitivity to the antigen used to produce the mediator (4). Supernates were tested at one-third or one-sixth dilutions in medium TC-199 containing 10% horse serum. The indicator lymphocytes were cultured for 6 days at 37°C at 2×10^5 cells/0.2 ml/well in quadruplicate in microtiter plates (Cooke Laboratory Products, Cooke Engineering Co., Alexandria, Va.). 18 h before harvesting the cultures, 1 μCi of [^{3}H]thymidine (New England Nuclear, Boston, Mass.) with a sp act of 6.7 mCi/mmol was added to each well and the acid precipitable material collected in a MASH II apparatus (Microbiological Associates, Inc., Bethesda, Md.). The radioactivity was measured in a Tri-Carb liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.) and the mitogenic activity was calculated as a stimulation index (SI = P/R) and a net increase in counts per minute (P − R).

Cell Treatment with 5-Bromo-2-Deoxyuridine (BUdR) and Light. In previous experiments it was shown that unseparated populations of antigen-stimulated lymphocytes treated with BUdR and light were capable of producing MIF despite the finding that over 95% of the proliferative response in the cultures was suppressed (17). Dividing lymphocytes treated in this manner are thought to be destroyed after several cycles of division but direct evidence of this premise is lacking. These experiments were interpreted to mean that nondividing lymphocytes were producing MIF. Since unseparated populations were used previously, it was of interest in the present study to repeat these experiments using purified populations of T and B cells. Unseparated and T- or B-cell populations were cultured in medium TC-199 containing 15% horse serum and 10^{-4} M BUdR. Cells were incubated with or without antigen (PPD, *Candida*, or SK-SD) and received an exposure to visible light (1 h) on days 2, 3, and 4. After 6 days of culture the cells were washed and recultured for another 48 h in the presence of the same antigen. The supernates were collected and assay for MIF activity.

Results

Production of MIF by T- and B-Cell Populations. The data indicate that both T- and B-cell populations derived from the blood of individuals exhibiting cutaneous delayed hypersensitivity to PPD, *Candida*, or SK-SD produced MIF when stimulated in vitro by these antigens (Fig. 1). In three experiments using cells from PPD-positive donors, supernates from antigen-stimulated T or B cells inhibited macrophage migration by an average of 23% and 29%, respectively. No MIF was produced in three experiments using lymphocytes from PPD-negative donors; supernates from T- and B-cell populations inhibited macrophage migration by −3% and 5%, respectively. Similar results were obtained in experiments where *Candida* and SK-SD were used as antigens.

It was then of interest to quantitate the amount of MIF produced by each subpopulation. Cultures were set up with 5×10^6 T or B cells, with and without antigen, and incubated for 48 h. The supernates obtained from these cultures were serially diluted, and the highest dilution which still contained MIF activity was determined. It can be seen in Fig. 2 that MIF produced by B cells was still present at a titer of 1:4 whereas T-cell MIF activity was only detected in the undiluted supernate.

Chromatography of Human T- and B-Cell MIF. Since it is well known that antigen-antibody complexes can inhibit macrophage migration (18), it was important to further characterize the inhibitory activities obtained from each cell population. In three experiments, the supernates from stimulated and control cell cultures were filtered over Sephadex G-100 columns, and the fractions were assayed for MIF activity on guinea pig macrophages (Fig. 3). It can be seen that peak activity of both T- and B-cell MIF were found in the same fraction (IVb, 23,000 daltons). The mean T-cell MIF inhibitory activity was 32% and that for

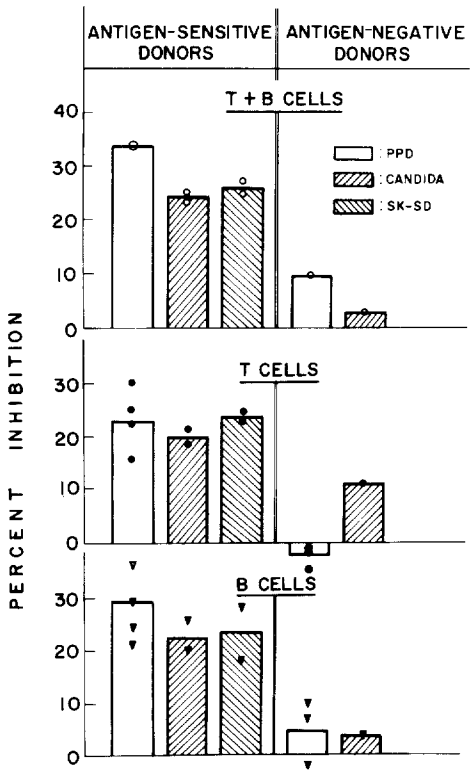

FIG. 1. MIF production by T and B cells. Inhibition of migration of guinea pig macrophages by supernates obtained from different cell populations. Bars indicate mean MIF activity and symbols represent individual experiments. Note that MIF is produced by T and B cells but only when sensitive donors were tested.

the B cell was 36%. Human MIF obtained from unseparated lymphocyte populations has previously been shown to elute in the same fraction (15). In one out of three experiments, MIF activity was also found in fraction IVa but in none of the other fractions. These studies indicate that inhibition was not due to antigen-antibody complexes which elute, because of their size, in fractions I and II.

Effect of BUdR and Light on MIF Production by T and B Cells. It was shown in previous studies that antigen-induced MIF could be elaborated by a population of nondividing lymphocytes (17). Furthermore, Chess et al. have demonstrated that T cells but not B cells proliferate directly in response to antigen (11). These findings taken together suggested that B lymphocytes could be the nondividing cells which were producing MIF. Experiments were carried out to examine this hypothesis and to determine whether or not the T cells which produce MIF are proliferating cells.

Treatment of a population of antigen-stimulated lymphocytes with BUdR and light eliminates the dividing cells: cells undergoing antigen-induced proliferation incorporate BUdR into their DNA and are subsequently damaged and eliminated when exposed to light. As is shown in Fig. 4, MIF was still produced by

unseparated lymphocyte populations pretreated with antigen, BUdR, and light in five of six experiments (mean 21% inhibition). In contrast, T-cell populations similarly treated did not produce MIF (mean 8% inhibition). The control population of T cells which were not exposed to antigen initially, although pretreated with BUdR and light, did produce MIF (mean 21% inhibition). In contrast, in two experiments, B cells pretreated with antigen, BUdR, and light behaved similarly to the unseparated population, i.e., they were not inhibited from producing MIF.

Production of LMF by T-Cell Populations. Antigen-stimulated lymphocytes from sensitized donors elaborate a mitogenic factor which induced nonsensitized lymphocytes to incorporate [^{3}H]thymidine. To determine which cell type produces LMF, column-purified lymphocyte populations were obtained from both PPD or *Candida*-sensitive and -nonsensitive subjects. In each of these experiments, the unseparated lymphocyte population, purified T cells and purified B cells were incubated in the presence or absence of the appropriate antigen. The supernates from these cultures were then assayed on indicator cells from individuals lacking sensitivity to the antigens being tested. In every case, there were three sets of indicator cells: unseparated lymphocytes, T cells, and B cells.

Tables I and II illustrate the results of experiments using PPD and *Candida* as antigens. Unseparated sensitive lymphocytes stimulated by specific antigen produced a mitogenic factor which induced unseparated cells, T cells, and B cells to proliferate. Sensitive T cells from these individuals also produced mitogenic factor which caused a proliferative response in each group of the indicator cells. In contrast, antigen-stimulated B cells did not make LMF, although they did respond to it. The production of LMF, like MIF, was shown to be antigen specific; lymphocytes from PPD or *Candida*-negative donors stimulated by these

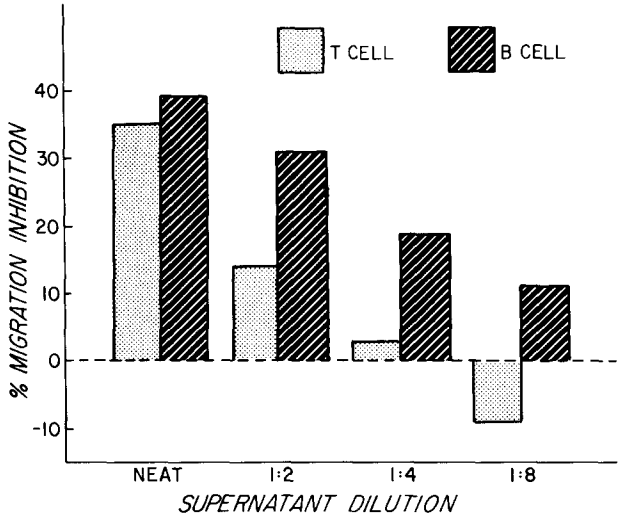

FIG. 2. Quantitation of MIF by T and B cells. Supernates from the lymphocyte cultures were serially diluted in culture medium. Note that B-cell MIF is still active at 1:4 whereas T-cell MIF is only active at NEAT.

antigens failed to elaborate a mitogenic factor for any of the indicator cells (Table III).

Chromatography of LMF from Human T Cells on Sephadex G-100. Antigen-antibody complexes can nonspecifically activate lymphocytes to increase their DNA synthesis. Therefore, it was important to further characterize the mitogenic activity present in the T-cell supernates.

In three experiments, supernates from PPD and *Candida*-sensitive donors were filtered on Sephadex G-100 and the fractions assayed for LMF using lymphocytes from negative donors (see Table IV). It can be seen that in each experiment the peak mitogenic activity was detected in fraction IVb (mol wt 23,000 daltons) which is the same region where MIF activity is also detected. Some mitogenic activity was also found in fraction IVa, the region between the albumin and chymotrypsinogen markers. It is of note that no mitogenic activity was found in fractions I and II, the region where antigen-antibody complexes are eluted.

Discussion

The studies described here were undertaken to evaluate the capacity of purified T and B lymphocytes to produce two mediators, MIF and LMF. The results

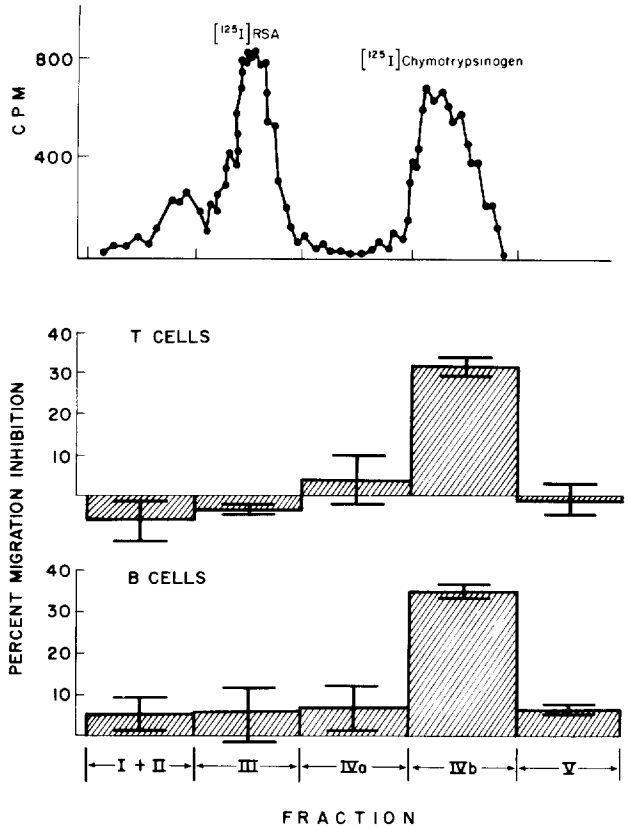

FIG. 3. Sephadex G-100 chromatography of MIF from T or B cells. Columns show MIF activity of 10 times concentrated fractions. Standard error of mean indicated.

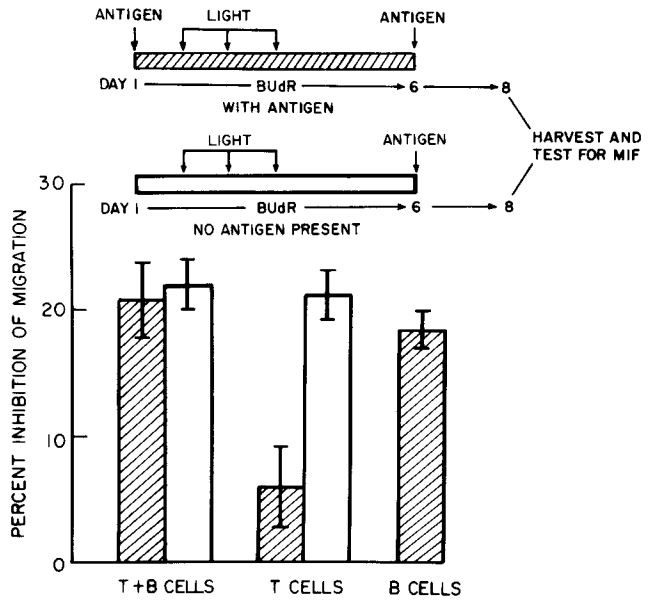

FIG. 4. Effect of BUdR and light on MIF production. Protocol for culture conditions is on the upper part of figure. Note that antigen-stimulated T cells are sensitive to BUdR and light treatment and do not produce MIF whereas T plus B cells and B cells alone are not.

indicate that both T and B lymphocytes produce MIF; indeed, B cells made more than T cells. In contrast, LMF was made by T cells but not B cells. These data necessitate a re-evaluation of the working hypothesis, heretofore held, that antigen-induced mediator production is the exclusive property of T lymphocytes.

Before discussing the implications of these findings, it is important to examine and evaluate the question: can the production of MIF by the B-cell population be due to a few contaminating T cells? The results previously described by Chess et al. using this technique (10, 11) combined with the data presented here virtually exclude this possibility; these are discussed below.

As described by Chess et al., the cells that do not adhere to the anti-Fab Sephadex column, the T-cell population, are 98% nonimmunoglobulin-bearing cells. These cells do not become immunoglobulin bearing when kept in culture for as long as 8 days, nor when incubated with human immunoglobulin or triggered by mitogens (10). Over 80% of these cells form E rosettes. On the other hand, the B-cell population, which is specifically eluted from the column with human Ig, contains 98% immunoglobulin-bearing cells, and less than 2% of this population form E rosettes. This population remains immunoglobulin bearing after culture. Both T- and B-cell populations contain approximately 2% monocytes as assessed by phagocytosis of latex particles.

The functional studies make it very unlikely that the cells in the B-cell population without B-cell characteristics are T cells responsible for the production of MIF in this population. First, the T-cell population responds to antigen by enhanced [^{3}H]thymidine incorporation and contains the responsive cells in the mixed leukocyte culture reactions; the B-cell population does not exhibit either of these functions (11). Second, the T-cell population contains the lymphocytes

TABLE I
Mitogenic Factor

Supernatant source (PPD positive)	Indicator cells (PPD negative)					
	T and B Cells		T Cells		B Cells	
	P-R*	SI‡	P-R	SI	P-R	SI
T and B cells						
1	1,155§	6.3	524	3.4	762	7.7
2	1,008	6.3	738	5.4	1,497	10.7
T cells						
1	1,437	8.5	499	4.1	1,373	11.5
2	1,598	10.4	1,002	8.0	1,533	8.3
B cells						
1	50	1.2	42	1.2	68	1.5
2	55	1.2	11	1.1	120	1.6

* cpm preincubated – cpm reconstituted (control).
‡ SI, P/R.
§ cpm, mean of quadruplicate cultures.

TABLE II
Mitogenic Factor

Supernatant source (*Candida* positive)	Indicator cells (*Candida* negative)					
	T and B cells		T cells		B cells	
	P-R	SI	P-R	SI	P-R	SI
T and B cells						
1	865	6.1	600	3.9	1,210	7.8
2	1,056	6.5	366	2.8	468	4.9
T cells						
1	1,345	9.5	674	5.2	1,440	5.1
2	.742	4.7	387	2.9	762	5.6
B cells						
1	−16	0.9	38	1.2	102	1.4

which are effectors in cell-mediated cytotoxicity, whereas the B-cell population does not.[2] Third, the T cells make LMF and the B cells do not (Tables I and II). Fourth, the MIF activity in the supernate from T cells is lost when diluted 1:2 while that from B cells is still present at 1:4 (Fig. 2). Fifth, the T cells do not produce MIF when pretreated with antigen, BUdR, and light, i.e. are proliferating cells, but the B cells still do, indicating that they are a different population which need not proliferate (Fig. 4). Thus, any contaminating proliferating T cells should have been eliminated from the B-cell population in these experiments.

[2] Sondel, P. M., L. Chess, R. P. MacDermott, and S. F. Schlossman. Immunologic functions of isolated human lymphocyte subpopulations. III. Specific allogeneic lympholysis mediated by human T cells alone. Manuscript submitted for publication.

Sixth, in previous studies, we found that MIF activity was not detected in supernates obtained from antigen-stimulated lymphocyte cultures which were made to contain as many as 10% lymphocytes from a sensitive donor, the remaining 90% coming from a nonsensitive donor (unpublished observations). It thus seems impossible to attribute the production of MIF by B-cell populations to a few contaminating T cells. The possibility that the B-cell MIF is dependent upon or directed by a few T cells which themselves do not make MIF would require the presence of a unique nonproliferating T cell exhibiting no other T-cell function; at present, this seems rather remote.

Our finding that T cells elaborate MIF when stimulated by specific antigen is in agreement with studies by Yoshida et al. who used guinea pig lymphocyte populations separated by rosetting techniques (19). On the other hand, our studies on B cells differ from these workers. They reported that B cells from specifically sensitized guinea pigs did not produce MIF in response to protein antigens and hapten-protein conjugates with the exception of PPD, and that B cells produced MIF in response to this antigen whether they had been obtained from a PPD-sensitive or -nonsensitive guinea pig. The authors speculate that in this instance, PPD was acting as a nonspecific B-cell mitogen. In contrast, in our studies, human B cells from nonsensitive donors did not produce MIF in response

TABLE III

Supernatant source (PPD negative, *Candida* negative)	Indicator cells (PPD negative, *Candida* negative)					
	T and B cells		T cells		B cells	
	P-R	SI	P-R	SI	P-R	SI
T and B cells						
PPD	65	1.3	20	1.1	21	1.2
Candida	2	1.0	7	1.1	28	1.2
T cells						
PPD	−3	1.0	50	1.3	31	1.2
Candida	15	1.1	9	1.0	46	1.2
B cells						
PPD	31	1.1	−18	0.9	22	1.1
Candida	42	1.2	−22	0.9	−13	0.9

TABLE IV

Mitogenic Factor

	Fraction (Sephadex G-100)				
	I and II	III	IVA	IVB	V
SI					
1	1.0	0.9	1.0	7.6	1.2
2	0.9	1.5	9.7	29.0	—
P-R (cpm)					
1	1,075	−4,357	39	120,862	2,205
2	−5	27	558	2,045	—

to three antigens, PPD, SK-SD, and *Candida*. Thus, we found that both T and B cells made MIF in response to several antigens, but only if the lymphocytes were obtained from specifically sensitive donors. It will be of interest to determine the functional properties of highly purified populations of guinea pig T and B lymphocytes obtained by affinity column techniques.

Recently, it has been reported that two other lymphocyte mediators, chemotactic factor (20) and interferon (21), are also produced by human B cells after being stimulated by PHA. Further, supernates from long-term lymphoid cell lines, many of them bearing surface immunoglobulin, have been reported to contain mediator-like activity including MIF (22, 23). Most long-term lymphoid lines originate from B cells.

Chromatographic analysis of T- and B-cell MIF indicate that neither activity is due to antigen-antibody complexes as the MIF produced by both cell types elutes from Sephadex G-100 in fraction IVb (23,000 daltons), the same location as MIF from unseparated human lymphocyte populations. Studies in which lymphocyte populations were pretreated with antigen, BUdR, and light are of special interest as they further differentiate the lymphocyte populations making MIF into one requiring proliferating cells (T lymphocytes) and one of non-proliferating cells (B lymphocytes). Cells which are induced to proliferate in the presence of BUdR incorporate the nucleotide analogue of thymidine into their DNA rendering the cell susceptible to damage and elimination by exposure to light. This procedure effectively eliminated the MIF-producing T cells, but not the MIF-producing B cells. It is important to recall that T cells, but not B cells, exhibit antigen-induced [^{3}H]thymidine incorporation (11). Thus, both antigen-sensitive and MIF-producing T lymphocytes are proliferating cells. The previous observation that some lymphocyte populations from patients with varying types of immunodeficiency contain cells that undergo antigen-induced proliferation but do not make MIF suggests the existence of at least two subpopulations of proliferating T cells, one of which does not make MIF.

It was of interest to determine which of the purified populations made more MIF. Quantitative determinations of the MIF activity in the present study indicate that B-cell populations make more MIF than T cells. This finding is consistent with the results of previous experiments in which pretreatment of unseparated lymphocytes (containing approximately 20% B cells) with antigen, BUdR, and light did not significantly diminish detectable MIF production (17).

Whereas MIF is produced by both T and B cells, LMF, is only made by T-cell populations. The production of LMF is antigen specific when PPD and *Candida* are used. Its effect, however, is nonspecific in that it increases the incorporation of [^{3}H]thymidine into lymphocytes from donors who lack sensitivity to the antigen used to produce it. In addition, LMF, when added to lymphocytes from specifically sensitized donors, causes more [^{3}H]thymidine incorporation than that caused by antigen alone.

LMF, although only produced by T cells, induces increased [^{3}H]thymidine incorporation by both purified T- and B-cell populations. Geha et al. have previously reported that enriched human T-cell populations isolated from discontinuous albumin gradients produce LMF which caused populations of enriched T or B cells to take up [^{3}H]thymidine (24, 25). They further stated that

their material induced antibody synthesis in B cells obtained from donors having sensitivity to specific antigen (25).

Chromatographic analysis of LMF indicates that its mitogenic activity is not due to antigen-antibody complexes as it too elutes from Sephadex G-100 columns in fraction IVb (23,000 daltons), the same location where MIF is found. Two lines of evidence suggest that LMF is quite distinct from MIF. First, B-cell MIF has no LMF activity. Secondly, some patients have lymphocytes which respond to antigen by producing LMF but not MIF (26). The existence of subpopulations of lymphocytes making separate and distinct mediators most easily explains these findings. If one argues that they are the same molecule, it is then necessary to postulate the production of regulatory substances which can inhibit one activity but not the other. This hypothesis seems unlikely at this time, but it is subject to analysis.

At this juncture, it is necessary to re-examine the interpretation of certain studies carried out with peripheral blood lymphocytes from patients with immunodeficiency diseases in the light of the present findings. The lack of antigen-induced MIF production by lymphocytes from patients with thymic aplasia using such common antigens as SK-SD and *Candida* (7) now implies both a T- and B-cell defect. The lack of a proliferative lymphocyte response to PHA has similar implications. Such patients are capable of mounting certain antibody responses and thus some B-cell function is intact. Since the defect in these patients is overcome by thymus grafting, it would appear that the ability of T and B cells to become sensitized to antigen or to develop responsiveness to mitogen is still a thymus-dependent process.

Results from patients with chronic mucocutaneous candidiasis present a greater paradox. Many such patients exhibit normal T-cell function as assessed by [^{3}H]thymidine incorporation in response to *Candida* antigen, and normal B-cell function measured by high titer antibody to the same antigen (27). Their cells also respond normally to PHA. However, in many cases, their lymphocytes do not make MIF in response to *Candida* antigen, implying a defect in both T and B cells, and further, these patients do not exhibit cutaneous delayed hypersensitivity to this antigen (28). Clearly, there exists a multiplicity of subpopulations of *Candida*-sensitive T and B cells; the possible requirement for both in the manifestation of cutaneous delayed hypersensitivity needs to be further elucidated.

How can the finding that B cells make antibody to certain antigens but do not produce MIF when stimulated by the same antigen be explained? Possibly, there are subpopulations of B cells. Further, a continuous T-cell suppression of such B-cell responses might exist. It would be of interest to determine if B cells from such individuals could make MIF if the T cells were removed.

The observation that B cells make MIF but do not proliferate in response to antigen may explain the finding that lymphocytes from some patients with Wiskott-Aldrich syndrome who are treated with transfer factor produce MIF but do not proliferate in response to antigen (29). In these cases, transfer factor may be turning on the B lymphocytes, but not the T cells. On the other hand, such patients, after transfer factor, exhibit positive cutaneous delayed hypersensitivity. Could this be a B-cell manifestation in this case? It would appear that the

relationship of T and B lymphocytes to cutaneous delayed hypersensitivity in man requires re-exploration.

At present, no single in vitro assay of lymphocyte function correlates completely with in vivo cutaneous delayed hypersensitivity. Antigen-mediated [^{3}H]thymidine incorporation (11) and LMF production clearly reflect in vitro T-cell function; however, in some diseases such as in sarcoidosis or ragweed allergy, these responses may be positive in the absence of cutaneous hypersensitivity or MIF production (8, 26). As both T and B cells produce MIF, it cannot be considered an assay of T-cell function in man. It should be emphasized, however, that lymphocytes must be obtained from donors exhibiting delayed hypersensitivity to the antigen used to elicit MIF production. Thus, with some exceptions, MIF production is associated with the presence of delayed hypersensitivity, but not with a particular cell type. Clearly, the manifestations of delayed hypersensitivity in man cannot be explained completely by the present schemes and require further investigation.

Summary

Highly purified populations of T and B lymphocytes obtained by affinity column separation were stimulated by antigen and their ability to produce two mediators, migration inhibitory factor (MIF) and lymphocyte mitogenic factor (LMF) was assessed. Both T- and B-cell populations made MIF; the production of MIF was antigen-specific using purified protein derivative of tuberculin, streptokinase-streptodornase, and *Candida* antigens. The MIF activity from both populations could not be attributed to antigen-antibody complexes as the inhibitory activity eluted from Sephadex G-100 columns in the same region corresponding to mol wt 23,000 daltons. Further studies indicate that the T cells producing MIF are proliferating cells whereas the B cells producing this mediator are not.

In contrast, LMF was made only by T cells and not B cells when these populations were stimulated by antigen. The LMF induced the [^{3}H]thymidine incorporation into both T and B cells obtained from donors lacking sensitivity to the antigens used to elicit the factor. Chromatographic studies indicate that LMF eluted from Sephadex G-100 in a fraction of mol wt 23,000 daltons where MIF is also found; however, since B cells produce MIF but not LMF, these two factors appear to be distinct from one another.

Some of the implications of these findings are discussed. The explanation for the production or lack of production of MIF by lymphocytes obtained from patients with immunodeficiency disorders requires reinterpretation.

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