PARKE-DAVIS
LECTURE

THE ROLE OF CELL-MEDIATED IMMUNITY IN THE INDUCTION OF INFLAMMATORY RESPONSES

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The Role of Cell-Mediated Immunity in the Induction of Inflammatory Responses

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Reactions of cell-mediated immunity fall into two broad categories: those that involve direct participation of intact lymphocytes in the effector mechanism of the reaction and those that involve mediation by soluble lymphocyte-derived factors known as lymphokines. The first kind of reaction is essentially limited to lymphocyte-dependent cytotoxicity, although certain aspects of T cell–B cell cooperation may fall into this category as well. The second category appears to comprise the bulk of the so-called cell-mediated immune response and provides a link between this system and the inflammatory system. Various lymphokines have been shown to exert profound influence upon inflammatory cell metabolism, cell surface properties, patterns of cell migration, and the activation of cells for various biologic activities involved in host defense. Although substantial information is now available about various physicochemical as well as biologic properties of lymphokines, purification and characterization data are as yet too incomplete to allow us to ascribe all of these activities to discrete mediator molecules. Current work involving the development of antibody-based techniques for mediator assay may shed light on this issue. Information on the kinds of cells capable of lymphokine production is now available. Contrary to prior expectation, T cells are not unique in their capacity for lymphokine production. Under appropriate circumstances, B cells and even nonlymphoid cells can do so as well. The unique property of lymphocytes in this regard appears to relate to their ability to respond to certain specialized signals such as specific antigen or an appropriate mitogen. Mediator production per se may represent a general biologic phenomenon. Although lymphokines have been defined mainly in terms of *in vitro* assays, early speculations about their *in vivo* importance are proving correct. Evidence for the role of lymphokines comes from studies involving detection of lymphokines in tissues, studies involving injection of exogenous lymphokines, and studies involving suppression of *in vivo* reactions by

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various techniques. The use of antilymphokine antibodies has proven useful in the latter kinds of experiments. Work in many laboratories is beginning to relate these findings to clinically relevant situations. A major unsolved problem relates to the regulation and control of lymphokine production and activity. At present only a limited body of information is available on this point. This is a potentially fruitful area for future investigation since it may provide techniques for manipulating the immune system in ways that are clinically useful. (Am J Pathol 88:501–528, 1977)

IN SPITE OF AN ENORMOUS BODY of work on the subject, the concept of cell-mediated immunity remains a puzzling one. Depending upon ones' research interests or personal bias, the phrase conjures a limited vision of either lymphocyte-dependent cytotoxicity on the one hand or the classic cutaneous tuberculin-type delayed hypersensitivity reaction on the other. Even the names represent misnomers. The reaction is not delayed, at least, not in comparison with lesions mediated by reaginic antibody which have latent periods of 48 to 72 hours. Moreover, the classic time course of 24 to 48 hours for delayed hypersensitivity is merely a function of its traditional site for elicitation, the skin. As we shall see, in other locations such reactions may reach maximal intensity in as little as 4 hours. Moreover, this class of immunologic reaction is no more or less cell-mediated than antibody-dependent reactions which are ultimately due to the participation of a lymphocyte or plasma cell. With the exception of those reactions which involve direct cell killing, most reactions of cell-mediated immunity are not dependent upon the lymphocyte as effector, but rather on soluble lymphocyte products known as lymphokines. Finally, "hypersensitivity" is obviously not a good way of describing a normal physiologic process.

It is even more instructive to look at a typical textbook definition of delayed hypersensitivity. It is usually defined as a slowly evolving reaction composed predominantly of mononuclear cells accumulating at the test site. The reaction is indurated, and this is said to be due to the intense cellular infiltration. It is capable of transfer by intact cells but not serum. An up-to-date text will usually mention that the reaction is T cell–dependent and that migration inhibition factor (MIF) released by the T cells locally plays a critical role. Although there is a sound basis for this general concept, current knowledge reveals it to be a great oversimplication which is accurate only under very limited circumstances. In fact, the one important principle that we can abstract from the definition is the relationship between cell-mediated immunity and an inflammatory response. It is this relationship that will be explored in the present discussion.

The Nature of the Cellular Infiltrate

Until little more than a decade ago, it was thought that the bulk of the mononuclear cells comprising the infiltrate of delayed reactions represented sensitized cells possessing some sort of immunologic specificity directed toward the antigen and that there was some mechanism that served to attract such cells to the reaction site. However, it is now known that only a small number of infiltrating cells are specifically sensitized cells. Moreover, the bulk of the available evidence suggests that there is little, if any, preferential accumulation of these sensitized cells at the specific test site. The evidence for these contentions is based mainly on transfer experiments; these have been extensively reviewed.[1] Another kind of experiment, which does not depend upon labeling of a pool of donor cells of differing specificities, is illustrated in Table 1. In this study,[2] guinea pigs were immunized with two unrelated antigens at different times. ^{3}H-Thymidine was administered in such a manner that it only labeled cells proliferating in response to the first antigenic stimulation. Skin tests were performed with each antigen at separate sites 10 days after the administration of the first antigen. As can be seen in Table 1, there was

Table 1—Percentage of Labeled Mononuclear Cells in Skin Reactions of Guinea Pigs Immunized With Two Antigens and Given ^{3}H-Thymidine After the First

Guinea pig No.	OCBC	TAT
1	13.5	5.0
2	3.2	7.2
3	12.2	1.1
4	6.2	4.9
5	8.4	8.4
6	5.3	9.2
7	5.0	4.4
Mean	7.7	5.7
Standard deviation	1.4	1.0
	TAT	OCBC
8	7.5	8.3
9	2.3	2.7
10	5.3	7.7
11	6.3	6.1
12	4.7	2.1
13	5.0	5.1
14	2.6	1.5
Mean	4.8	4.8
Standard deviation	0.7	0.9

Guinea pigs 1 through 7 were immunized with OCBC on Day 1, given ^{3}H-thymidine on Days 1 to 4, and immunized with TAT on Day 6. In guinea pigs 8 through 14, the reverse sequence of antigens was used. OCBC = *o*-chlorobenzoyl chloride, TAT = diphtheria toxoid antitoxin. This table was reproduced wih permission from J Immunol 98:269-273, 1967.

no significant difference in the small number of labeled cells at each reaction site, regardless of which antigen was given first. Comparison of the percentage of labeled cells in each test site in individual animals showed that of the 14 animals studied, 7 had higher labeled cell counts to the first antigen, 6 had higher counts to the second antigen, and 1 showed no difference. Thus, there is no evidence for preferential accumulation of specifically sensitized cells at either site.

Another misconception about the nature of the inflammatory response is related to the indurated nature of the delayed hypersensitivity reaction site. This has been considered to be a macroscopic reflection of the vast numbers of infiltrating inflammatory cells. Some time ago, however, it was shown by ourselves [3] and by Nelson [4] that one could suppress cutaneous delayed hypersensitivity reactions by treating the experimental animals with anticoagulants. We found that this suppression involved only the effector arm of the response and not the state of immunization. The suppression was noted mainly as a decrease in induration; histologic observation utilizing sections routinely stained with hematoxylin and eosin demonstrated substantial mononuclear cell infiltration in the suppressed animals. Moreover, the cellular inflammatory response to nonspecific irritants was not affected in these animals. These early studies have been brought into focus by the elegant studies of Dvorak and his associates.[5] Using modern morphologic investigative techniques, they have unequivocally demonstrated deposition of fibrin in delayed hypersensitivity sites. In comparative studies involving nonindurated, basophil-rich, cell-mediated lesions, they have shown that the induration of delayed hypersensitivity reactions is not due to the infiltrating cells but rather is due to the deposition of fibrin, possibly in association with bound extravascular water.

Even the nature of the cellular inflammatory infiltrate itself is not as clear-cut as is generally believed. It is certainly true that the typical cutaneous manifestations of cell-mediated immunity in the guinea pig and man is a lesion that is composed predominantly of mononuclear cells, with macrophages representing the great majority of these cells and lymphocytes present in lesser numbers. However, in both the guinea pig and man, it is possible to induce cell-mediated immune lesions in which the predominant cell is the basophil. These reactions, known as Jones-Mote hypersensitivity or cutaneous basophil hypersensitivity (CBH) occur when the immunizing stimulus is weak, for example, when antigen is incorporated into incomplete Freund's adjuvant. Also, many contact allergies of man seem to be of the CBH variety.

In certain circumstances, the eosinophil may play an important role. If

one skin-tests a guinea pig, allows the reaction site to heal, and then reinjects antigen at that site, then the second reaction is more intense, evolves more rapidly, and is largely composed of eosinophils. This is known as a "retest" reaction.

Even the classic delayed hypersensitivity reaction may have a different morphologic appearance in different species. For example, in the mouse,[6] the lesion contains large numbers of neutrophils. Finally, in certain experimental autoimmune disorders in which cell-mediated immunity is thought to play a pathogenetic role (such as thyroiditis or encephalomyelitis) the predominant infiltrating cell appears to be the lymphocyte.

These examples demonstrate that the typical macrophage-rich reaction represents but one manifestation of delayed hypersensitivity reactions. The central feature common to all is that a small number of specifically sensitized lymphocytes interact with antigen locally. As a consequence of that interaction, a set of events occurs which leads to the generation of an inflammatory response at the reaction site. Those events are known to involve the production and release of soluble mediator substances that are collectively referred to as lymphokines.

The Lymphokines

Migration Inhibition Factor

Initially, delayed hypersensitivity reactions such as those described in the previous section were the focus of most of the studies aiming toward the elucidation of the mechanisms involved in this class of immunologic phenomena. The subsequent discovery of a variety of assay systems that represented *in vitro* correlates of delayed hypersensitivity markedly extended the range of experiments that could be performed and thus led to much of our current information in this field.

In vitro studies on delayed hypersensitivity were initiated by Rich and Lewis, who demonstrated that tuberclulin preparations inhibited the migration of cells from spleen fragments or peripheral blood taken from actively immunized animals. This assay was simplified by George and Vaughan, who developed the modern capillary tube method. Bloom and Bennett and David independently demonstrated that the inhibition of migration of peritoneal exudate magrophages from such tubes was due to the release of a soluble factor (MIF) from sensitized lymphocytes reacting with antigen in the exudate suspension. The migration inhibition reaction could be demonstrated using peritoneal exudate cells from nonimmunized animals, provided that a source of MIF was included in the incubating mediums. The best source proved to be supernatant fluids from cultures of

sensitized lymphocytes incubated with specific antigen. These early studies are discussed and their references cited in a recent review.[7]

Migration inhibition factor is of great historical importance since it was the first of the nonantibody, lymphocyte-derived soluble factors to be described. It was the first lymphokine. Several important general properties of this class of mediator were first discovered in studies of MIF. These factors are of relatively low molecular weight as compared to conventional immunoglobulins, and they lack immunologic specificity. In other words, although the lymphokines are generated by specific immunologic reactions between antigen and sensitized lymphocytes, once formed they themselves do not require interaction with antigen for the expression of their biologic activity. Thus, they are not antibody-like. The few known situations (to be discussed later) in which mediators have apparent specificity are not exceptions to this role; in those circumstances, the specificity is due to the association of antigenic fragments with the mediators.

Three important methodologic principles were explicitly formulated in the early studies of MIF. First, control supernatants, which were obtained from the sensitized cells incubated in the absence of antigen, were always reconstituted with antigen prior to use. Second, controls for viability were also included. Third, reversibility of effect was demonstrated. By this is meant that if the migration-inhibiting preparations are allowed to remain in culture, the cells escape from inhibition and ultimately begin to migrate. These last two principles are of great practical importance. As a simple *reductio ad absurdum*, anything that kills a macrophage will prevent it from migrating. Since it is only in the very recent past that lymphokines have begun to be characterized and identified in precise physicochemical ways, the distinction between toxicity and migration inhibition remains a very important one to make. A surprisingly large number of experiments in the literature are difficult to interpret because this distinction was not always appreciated.

Kinds of Lymphokine Activities

For the most part, the lymphokines have been defined in terms of *in vitro* bioassays. They fall into three main categories: lymphokines that damage their target cells (lymphotoxins), lymphokines that cause cell proliferation (mitogenic factors), and lymphokines that modulate the inflammatory response. In the first category are included not only factors that kill or injure cells, but also factors that suppress certain of their biologic activities. One such example is *proliferative inhibitory factor* (PIF). Certain "helper" substances involved in T cell–B cell cooperation

may fall into the second category; MIF, already described, is a member of the third category.

Inflammatory Lymphokines

The best studied of the lymphokines involved in inflammatory responses are those that affect macrophages. A number of such lymphokine effects are listed in Table 2. It can be seen that in general these fall into well-defined categories. Lymphokines have been described that affect the general metabolic properties of these cells. In addition, there are lymphokines that affect cell surface properties, lymphokines that affect the migration properties of the cell, and lymphokines that activate the macrophages for phagocytosis and killing functions. These activities have been exhaustively reviewed in a number of recent publications [7,8] and will not be discussed in further detail here. It is obvious, however, that MIF falls into the category of a lymphokine affecting cell migration properties. Another important lymphokine in this category is the *macrophage chemotactic factor* (MCF) first described by Ward *et al.*[9] By definition, chemotaxis is directed movement. A chemotactic factor converts random cell movement to enhanced movement in the direction of an increasing concentration gradient of the factor. The usual assay system involves the Boyden double chamber technique, in which the test substance is placed in the lower compartment and the target cell suspension in the upper compartment. The two compartments are separated by a micropore filter with pore size large enough to allow cell migration through the filter. The number of cells migrating through the filter after a given period of incubation in the test preparation is compared to the number migrating in a control prepa-

Table 2—Ways in Which Lymphokines Have Been Shown to Affect Macrophages

Cell metabolism
Glucose oxidation
Protein synthesis
Glucosamine uptake
Cell surface
Loss of cell coat
Electrophoretic mobility
Interfacial tension
Cell migration
Ameboid motility
Migration inhibition
Chemotaxis
Cell activation
All of above
Phagocytosis
Cell-mediated killing

ration not containing the test substance. Increased numbers of cells in the presence of the gradient are taken as evidence of a positive chemotactic response. An important additional control here is to include a chamber with test substance in *both* compartments to abolish the gradient and so exclude the possibility that one is detecting nonspecific enhancement of random motility in the presence of the test substance rather than true chemotaxis.

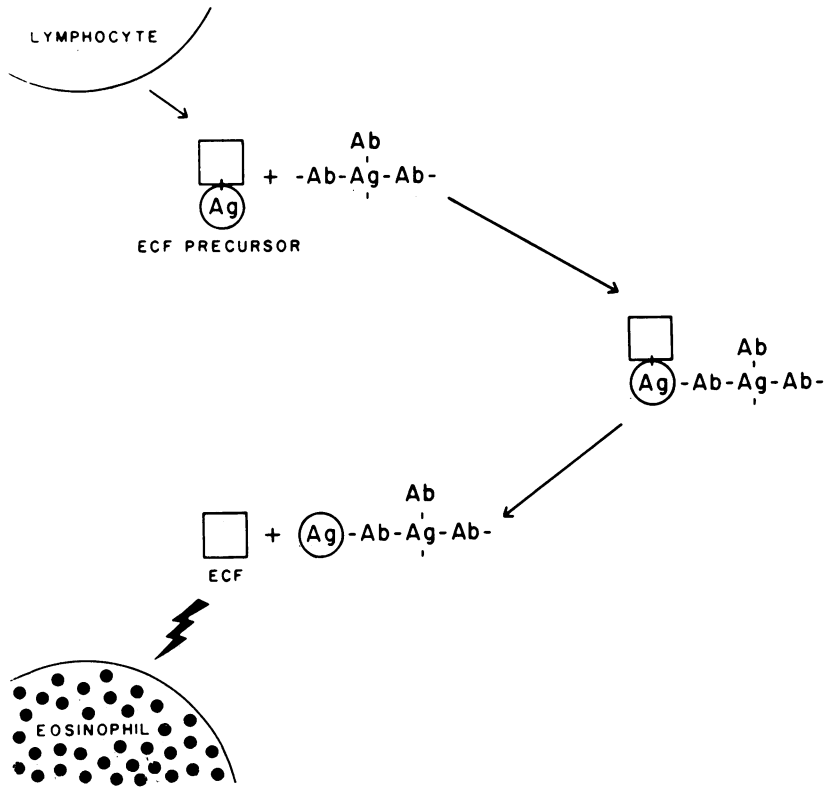
Since the above-mentioned lymphokine activities are defined in terms of bioassay rather than chemical identification, whether or not they are ascribable to discrete and therefore theoretically separable molecular species remains an open question. Certain lymphokines such as MIF and MCF appear to be different, but the case for others such as MIF versus *macrophage activating factor* (MAF) remains unclear.

The effects of lymphokines on other inflammatory cell types have been less well studied than their effects on macrophages. There is a migration inhibitory factor for neutrophils, LIF. This appears to be distinct from MIF, in part on the basis of inactivation studies using simple monosaccharides.[10] Also, there is at least one chemotactic factor for each granulocyte type and for lymphocytes. These, mainly on the basis of indirect evidence, appear to be distinct from one another. One such lymphokine, the *eosinophil chemotactic factor* (ECF) has unusual properties which are worth describing in detail.

As background, it is worth recalling that eosinophils are conspicuous components of inflammatory infiltrates at sites of various kinds of immunologic reactions. They are found in the nasopharynx and bronchi of patients with allergic conditions such as hay fever or asthma, and in the intestinal tract as a consequence of parasitic infestations. In experimental situations, they are present in peritoneal exudates following multiple injections of foreign protein, in lymph nodes draining sites of antigen administration, in skin following injection of antigen–antibody complexes into normal animals, and in skin of delayed hypersensitive animals following multiple injections of antigen at the same site. In addition, eosinophils may be found in certain autoimmune lesions which involve cell-mediated immune responses, such as experimental autoimmune thyroiditis. All these observations (reviewed in Cohen *et al.*[8]) show a relationship between the eosinophil and the immune system, and the last two findings cited suggest that this relationship, in part, involves cell-mediated immunologic reactions.

The various observations described above led us to the suspicion that a lymphokine with chemotactic activity for eosinophils exists. Preliminary studies of MIF-rich supernatant fluids from antigen-stimulated lym-

phocyte cultures failed to detect such a factor. We soon discovered,[17,12] however, that these supernatants contained an inactive precursor substance (ECF_p) which could be activated by reaction with specific preformed immune complexes *in vitro* to generate a potent eosinophil chemotactic factor. Studies using antigen-coupled and antibody-coupled immunoadsorbent columns have demonstrated that ECF_p behaves as though it is associated with a "piece" of antigen and that its activation to ECF is associated with a loss of that piece. These findings have led to the model for activation shown in Text-figure 1. In this model, ECF_p combines with a free antibody site in the immune complex by coprecipitation. As a consequence of this interaction, a modified, active, antigen-free mediator molecule (ECF) is then released. It should be noted that ECF, on injection into guinea pig skin, produces an inflammatory reaction in which the predominant cell type is the eosinophil. We have presented



TEXT-FIGURE 1—Diagrammatic representation of the proposed interaction between eosinophil chemotactic factor precursor (ECF_p) and immune complexes to generate ECF activity. (Reprinted from J Immunol 111: 1450–1458, 1973, with permission.)

indirect evidence that suggests that ECF may play a role in the cutaneous "retest" reaction [13] and in experimental autoimmune thyroiditis.[14]

Kinds of Cells That Make Lymphokines

Lymphocytes

On the basis of much indirect evidence, mostly based upon the fact that cell-mediated immune reactions appear to be manifestations of T lymphocyte function, it was assumed that T cells were the source of all lymphokines. Indeed, this was one of the major unquestioned answers of lymphokine biology. In 1973, Dr. Takeshi Yoshida, Dr. Hidekichi Sonozaki, and I explored this problem using surface markers such as the complement receptor on lymphocyte membranes as a basis for cell separation. Guinea pigs were immunized to a variety of antigens. A typical experiment is shown in Table 3. In all cases, T cells could make MIF, as expected. In the case of antigens such as egg albumin (EA) or bovine serum albumin (BSA), either unconjugated or conjugated with dinitrophenyl (DNP), B cells could not make MIF, again as expected. To our surprise, when purified protein derivative was used, B cells as well as T cells made MIF.[15] A variety of controls demonstrated that the observed activity could not be due to the contaminant T cells in the B cell preparations.

We next looked at suspensions of T and B cells from nonimmunized guinea pigs. The results are shown in Table 4. In this situation, T cells do not make MIF, confirming the nonimmune status of the experimental animals. As before, EA cannot induce B cells to make MIF. However, the B cells once more responded to purified protein derivative of tuberculin (PPD) with MIF production. In all cases, B cell–derived MIF and T cell–derived MIF were identical by all available physicochemical and

Table 3—Migration Inhibition Factor Production by Sensitized T and B Lymphocytes

Antigen*	Lymphocytes†	
	B cells	T cells
EA	−2.6 ± 7.2‡	30.0 ± 2.4‡
DNP-EA	−6.4 ± 4.2	28.1 ± 3.0
DNP-BSA	7.6 ± 8.4	42.0 ± 5.6
PPD	35.1 ± 5.9	30.9 ± 8.4

EA = egg albumin, DNP = dinitrophenyl, BSA = bovine serum albumin, PPD = purified protein derivative.

* Antigen in culture, 50 μg/ml.

† Cell concentration, 6×10^6/ml.

‡ Percent inhibition = $(1 - \text{area}_{exp}/\text{area}_{cont}) \times 100$.

Adapted from J Exp Med 138:784–797, 1973, with permission.

Table 4—Migration Inhibition Factor Production by Purified Protein Derivative Stimulation of Lymphocytes Obtained From Normal Animals

Lymphocyte source	Antigen*	B Cells†	T Cells†
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 × 10^6/ml.
‡ Percent inhibition.
Adapted from J Exp Med 138:784-797, 1973, with permission.

biologic characterization procedures. These results were put into perspective for us by the report that commercial preparations of PPD are B-cell mitogens. We repeated these experiments using endotoxin lipopolysaccharide (LPS), another known B-cell mitogen with identical results. These results have been confirmed in many laboratories. Thus, the capability of human B cells to make MIF; [16] of human [17] and guinea pig [18] B cells to make MCF as well as MIF;[19] and of human B cells to make (mitogen-induced) interferon [20] have all been recently described. In most of these studies, a requirement for B-cell activation by mitogenic factors has been demonstrated, although in one report [16] specific antigen was found capable of serving as inducing agent as well.

In retrospect, studies demonstrating MIF-like activity in long-term lymphocyte cultures may have detected a similar effect since some of those lines may have been B cell–derived. We explored this by specifically looking at a number of B and T cell–derived lines and found that both were capable of spontaneous MIF production.[21] They also had strong neutrophil chemotactic factor (NCF) activity.

The reports by ourselves and others that B cells are capable of producing MIF as well as other lymphokines raise an interesting paradox. The evidence for the association of cellular immunity and T-cell function is overwhelming. There is also good evidence that many, if not all, of the manifestations of cell-mediated immunity are due to lymphokine-dependent mechanisms.[8] Since B cells can make lymphokines *in vitro*, it is at first glance difficult to understand the apparent requirement for T cells in cell-mediated immunity. Part of the explanation for this paradox lies in the difference in the requirements for lymphokine induction by T and B cells. T cells can be activated by either specific antigen or mitogens. B cells, with the exception of one report to the contrary,[16] appear to require mitogenic activation. In those instances where specific antigen appears to induce B cells to produce MIF, this activity requires the simultaneous

presence of T cells,[22] and it may well be that under those circumstances that the T cells are providing an endogenous mitogenic factor.

This difference in the activation requirements for T and B cells cannot provide the whole explanation, since many substances such as PPD can function both as antigen and as B cell mitogen. One clue comes from the fact that the conditions under which B-cell lymphokine production occurs *in vitro* involve the use of purified subpopulations of lymphocytes, from which T cells are purposely removed. In our recent investigations, we therefore included cell mixing experiments. As before, lymphocytes were separated on the basis of the presence or the absence of a complement receptor. These cells, respectively CRL and NCRL, correspond to B cells and T cells, subject to the caveat that each procedure for separating cells based upon the exploitation of surface markers may lead to slightly different subsets of these broad categories. In our hands, these techniques lead to greater than 90% purity of B cells and greater than 95% purity of T cells. Since we can use nonimmunized animals as cell donors, pure populations of T cells are incapable of generating MIF on exposure to LPS or PPD, and so contamination of B cells by some T cells is not a problem in these studies.

In the first set of experiments, we obtained lymphocytes from normal guinea pig spleens and separated them as before. We found that incubation of 5×10^6 B cells or 1×10^7 B cells/ml with either PPD or LPS gave no significant difference in MIF production. This allowed a meaningful comparison between B cells at 1×10^7/ml and 50:50 mixtures of T and B cells, each at 5×10^6/ml, and avoided the necessity for differences in total cell concentrations in the various cultures.

The results of the experiment are seen in Table 5. As before, normal B cells, incubated with either LPS or PPD, generated MIF. T cells under these conditions gave activity well within background range. Adding T cells to the B cells totally removed their ability to make MIF. In these

Table 5—Effect of T Cells on B Cell-Derived Migration Inhibition Factor Activity

Lymphocytes*	Percent migration inhibition	
	PPD	LPS
T cells	6.2	2.5
B cells	30.5	29.7
T & B cells	7.0	3.8
Unseparated suspension	2.5	1.7

Purified protein derivative (PPD) and lipopolysaccharide (LPS) were the agents used to activate cells.

* Cells were obtained from nonimmunized animals and fractionated as described in the text. Total cell concentration 1×10^7/ml.

experiments, it should be noted that the original, unseparated lymphocyte suspensions were also incapable of MIF production.

These results suggested that we were dealing with a new class of T-cell suppressor function. This effect might be exerted either by direct cell–cell interaction or indirectly by a soluble factor. To explore this question, we incubated normal T cells in the presence or absence of PPD. The control cultures were reconstructed with PPD prior to use in the usual manner. Such experimental or control supernatants are devoid of MIF activity. B-cell suspensions were incubated with PPD, with or without either experimental or control T-cell supernatant. We found that the experimental, activated T-cell supernatant (but not the control) destroyed the ability of the B cells to make detectable MIF. Similar results were obtained with LPS-induced activation. This suggested that the T-cell suppressive effect was mediated by a soluble, T cell–derived factor. By definition, this represents yet another lymphokine which we have defined as MIFIF (MIF inhibition factor). MIFIF appears unique, not only in terms of its suppressive effect on B-cell mediator production, but also because it is the product of a T cell that is activated by a putative B-cell mitogen. The mechanism of this effect remains to be elucidated. MIFIF could either act directly on the B cells to prevent synthesis or release of MIF or might even be cytotoxic for those cells. A alternative possibility is that it could interact with the MIF as it is formed. However, preliminary evidence obtained by incubating MIFIF-containing supernatants directly with MIF-rich supernatants has shown that MIFIF does not appear to act directly on MIF or to compete with it for a receptor site on its target cell. Also, there is no evidence for cytotoxic effect. Thus, the first alternative appears likely.

Although a number of prior studies have provided evidence for the regulation of various manifestations of cell-mediated immunity by suppressor cells, MIFIF appears to be the first example of suppressor activity with respect to the lymphokines themselves. It may well be that mechanisms similar to those described here will be found operative with respect to conventional T cell–derived MIF as well and that these mechanisms play an important role in the regulation of cell-mediated immune reactions *in vivo*.

Nonlymphoid Cells

Work in our laboratory has demonstrated that the infection of a variety of cells by viruses such as mumps and Newcastle disease virus *in vitro* or *in vivo* and simian virus 40 (SV40) *in vitro* leads to the production of lymphokine-like substances which we have defined as cytokines (reviewed in Cohen[6]). Similar data has been obtained by others in the SV40 system.[23]

The mediators from these sources have strikingly similar physicochemical properties to those lymphokines with corresponding biologic properties. As will be discussed later, it is possible to prepare antisera against culture supernatants obtained from antigen-activated cultures of sensitized lymphocytes.[24,25] We found [26] that sepharose bead columns conjugated with an antibody prepared against lymphocyte-derived MIF were capable of adsorbing cytokine MIF (derived from SV40-infected African green monkey kidney cells). Columns conjugated with an antibody prepared against control supernatants had no such effect. Moreover, we found that cytokine MIF could substitute for lymphocyte-derived MIF in certain *in vivo* model systems involving desensitization. These results all suggest the identity of MIF from virus-infected nonlymphoid cells to conventional MIF. Further, they raise the interesting possibility that lymphokine production per se may be a general biologic phenomenon and that what is unique to the immune system is the way in which lymphoid cells can be activated for such production either by mitogen or specific antigen.

In Vivo Activity of Lymphokines

As has already been alluded to, very little is known about the fine details of lymphokine structure and chemistry. All of the lymphokines have been defined in terms of complex, semiquantitative biologic assays. Thus, although it is widely held that the lymphokines are the mediators of many of the manifestations of cellular immunity *in vivo*, it is not surprising that direct evidence for this contention has been difficult to obtain. The initial demonstrations that MIF-rich supernatants could produce inflammatory infiltrates when injected into guinea pig skin provided evidence on this point, but the significance of those observations was unclear because of the essentially nonspecific nature of the inflammatory response. Many of the investigations performed by Dr. Takeski Yoshida and myself have addressed themselves to this issue. The following discussion will outline some of the approaches we have taken.

Macrophage-Disappearance Reaction

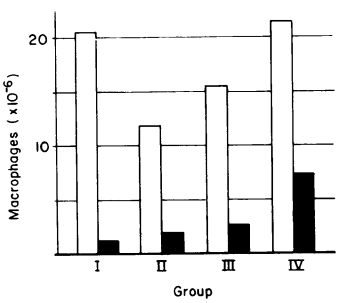
One can readily induce a nonspecific peritoneal inflammatory exudate in delayed hypersensitive guinea pigs by the intraperitoneal injection of glycogen. Three or four days after the injection of this irritant, these exudates consist mainly of mononuclear cells, and of these, macrophages predominate. The intraperitoneal injection of specific antigen at this time causes a prompt reduction in the macrophage content of such exudates; 5 hours following antigen administration there is a drop of approximately 90% in the absolute number of these cells recoverable in the peritoneal

fluid. There is a slow recovery of macrophage content over the next 24 to 48 hours. This reaction is known as the *macrophage disappearance reaction* (MDR) and has been shown to be a manifestation of cellular immunity.

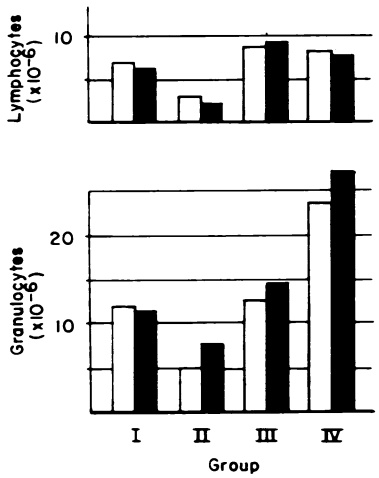
The MDR is a consequence of increased macrophage adhesiveness induced by specific antigen. This leads to clumping and sticking of macrophages to peritoneal surfaces, with a subsequent drop in the number recoverable from the peritoneal fluid. The bulk of the experimental evidence favors the view that the macrophage disappearance reaction is an *in vivo* analog of the migration inhibition reaction *in vitro*. It provides an especially suitable model system with which to explore lymphokine activity *in vivo*.

The results of our experiments [27] utilizing this model are summarized in Text-figure 2. Group I consists of animals undergoing an active MDR as described above. Group II consists of animals undergoing a passive MDR. These guinea pigs were not immunized. Rather, after the induction of the peritoneal exudate, they were given an intraperitoneal injection of sensitized lymphocytes (obtained from an immunized donor) and specific antigen. The control group received only lymphocytes and no antigen. This procedure gives a significant MDR. In Group III, the MDR was successfully transferred by lymphocytes enclosed in micropore chambers which remained cell-impermeable during the 5-hour time course of the experiment. In Group IV, the MDR was passively transferred using only cell-free MIF-rich supernatants from antigen-stimulated lymphocyte cultures. These supernatants were subjected to Sephadex chromatography to exclude the presence of immunoglobulins. Text-figure 3 demonstrates that none of these procedures had demonstrable effects on the granulocyte or lymphocyte content of the exudates.

Groups III and IV provide evidence that the MDR, which (as stated above) is a manifestation of cellular immunity, is mediated by a soluble, lymphocyte-derived factor.



TEXT-FIGURE 2—Macrophage disappearance reaction produced in various ways (control, *open columns*; antigen, *solid columns*): in actively immunized guinea pigs (20 μg antigen) (*I*); in nonimmunized recipients by peritoneally-derived sensitized lymphocytes (1×10^6 cells, 20 μg antigen) (*II*); in nonimmunized recipients by sensitized lymphocytes in micropore chambers (2×10^6 cells, 40 μg antigen) (*III*); and in nonimmunized recipients by a soluble, lymphocyte-derived factor (*IV*). (Reprinted from Cell Immunol 2:341–352, 1971, with permission.)



TEXT-FIGURE 3—The effect of various experimental procedures on the lymphocyte and granulocyte content of peritoneal exudates. The experimental groups are as defined in Text-figure 2. No significant effect on these cell populations is seen. (Reprinted from Cell Immunol 2:341–352, 1971, with permission.)

Extracts of Skin Reactions Sites

Many investigators have attempted to detect MIF activity in extracts of delayed hypersensitivity skin reaction sites. These attempts have been uniformly unsuccessful. There are several possible explanations for this failure, the most obvious one being that MIF might not play a role in the evolution of the delayed hypersensitivity reaction in the skin. Another and less heretical possibility is that only small amounts of MIF are produced and released at these sites and that this material is rapidly adsorbed onto cells or other tissue constituents. The adsorbed MIF might not be released into the medium by the relatively crude extraction procedures currently in use.

Our own efforts to find MIF activity in extracts of skin delayed reaction sites were also unsuccessful. However, armed with the knowledge that the lymphocyte culture supernatants could induce an MDR, we injected some of our skin extract fluids into nonimmunized guinea pigs bearing peritoneal exudates. To our surprise, we achieved a macrophage *appearance* reaction with a two- to threefold increase in macrophage content consistently observed. The MDR is thought to be an *in vivo* manifestation of MIF activity. Thus, it seemed likely that the anomalous macrophage accumulation caused by the skin extracts might be due to the chemotactic activity of MCF, acting under conditions where no MIF was available to induce cell stickiness and clumping. This expectation was confirmed [28] when the extracts of sites of delayed reactions to various protein antigens or of sites of contact reactions to *o*-chlorobenzoyl chloride, a potent sensitizing agent, were examined by means of the Boyden double chamber assay system for the presence of *in vitro* chemotactic activity. Chemo-

tactic activity toward macrophages and lymphocytes, but not neutrophils, could be detected in these extract fluids. Extracts prepared from normal skin or from skin sites of nonspecific inflammatory reactions did not show such activity. Physicochemical properties of these chemotactic factors were similar to those of respective factors previously obtained from supernatant fluids from lymphocytes cultured with specific antigen. Thus, at least two lymphokines, or at least lymphokine-like activities, could be recovered from sites of cell-mediated reactions.

Serum Lymphokine Activity in Experimental Animals

Yoshida *et al.*[29] have shown that rats of guinea pigs immunized with various antigens in complete Freund's adjuvant showed increases in peripheral blood monocytes for at least 2 weeks following immunization, in agreement with previous studies. However, when specific antigen was injected intravenously into such immunized animals, there was a prompt reduction in the number of circulating monocytes.

The maximal disappearance from the circulation occurred at 6 hours following antigen administration, and there was a slow return to normal over the next 24 hours. It was shown in these studies that the effect of antigen on blood monocytes was a function of the state of delayed hypersensitivity of the animals and that this reaction, like the MDR, was therefore a manifestation of cellular immunity.

Because of the analogies between this system and the MDR, Dr. Takeshi Yoshida and I attempted to detect MIF in the serum of such animals.[30] Guinea pigs were immunized by intramuscular injection of 0.5 mg of the BCG strain of tubercle bacilli and subsequently challenged intravenously with 0.5 mg of the BCG. This procedure induces an intense state of delayed hypersensitivity, and this state is associated with massive splenomegaly as well as peripheral lymphadenopathy. Sera were obtained from these animals at various times following intravenous challenge. Migration inhibitory factor activity was detectable in the sera of these animals but never in unimmunized animals or those controls that were immunized but unchallenged. Activity was maximal at 6 to 12 hours, corresponding approximately to the times of greatest reduction in the numbers of circulating monocytes.

Injection of Exogenous Lymphokines

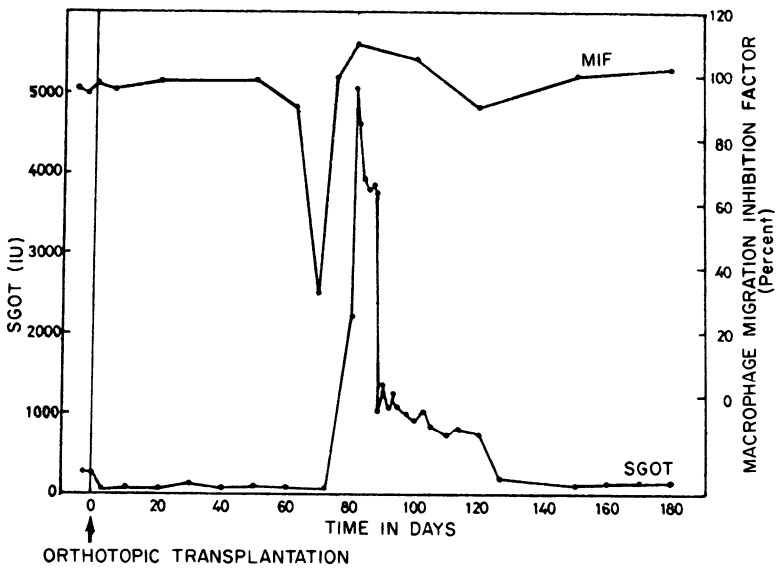
The detection of MIF in guinea pig serum following a systemic antigenic challenge in delayed hypersensitive animals and the temporal relationship between that serum MIF activity and the drop in circulating monocytes in such animals prompted a study of the consequences of

administering preformed, exogenous lymphokines to experimental animals.[30] We found that the intravenous injection of MIF-containing, but not control, supernatants into normal animals resulted in a drop in circulating monocytes comparable in magnitude to that observed in immunized animals challenged with specific antigen. Of even greater interest was the observation that lymphokine-treated animals developed anergy; if immunized animals were treated with lymphokines systemically, they became transiently incapable of mounting a skin reaction to the test antigen. Appropriate controls excluded participation of antigen or antibody in the suppressive effect. These results again confirmed that lymphokines could play *in vivo* roles.

Serum Lymphokine Activity in Man

The detection of MIF in the serum of experimental animals raised the possibility that MIF could be found in human sera during the course of certain diseases. The ability of exogenous MIF-containing supernatants to suppress cutaneous reactivity suggested that diseases associated with anergy might be good candidates. Accordingly, we initiated studies of patients with various lymphoproliferative disorders such as chronic lymphatic leukemia, multiple myeloma, Hodgkin's disease, non-Hodgkin's lymphoma, and the Sézary syndrome. We found that the large majority of such patients had detectable serum MIF.[31,32] MIF was thus found in B-cell disease as well as T-cell disease, in accordance with the observations already discussed on the ability of B cells to make lymphokines. Healthy individuals and patients with a variety of illnesses such as bronchopneumonia, adenocarcinoma of the colon, astrocytoma, diabetes, osteoarthritis, myelofibrosis, etc., have a combined incidence of detectable serum MIF of less than 2%. The other disease process in which we are now finding serum MIF with great frequency is sarcoidosis. It is tempting to speculate that in these conditions one explanation for the cutaneous anergy frequently observed involves the circulating lymphokine itself, in analogy with our studies in experimental animals.

One additional situation in which serum MIF is detectable is the posttransplantation hepatic dysfunction syndrome.[33] These patients have derangements in liver function that are often reflected in episodic variations in chemical parameters such as serum glutamic oxaloacetic transaminase (SGOT) levels. Such patients also have transient elevations in serum MIF activity, and the appearance of serum MIF invariably precedes elevations in SGOT. This is illustrated in Text-figure 4. The significance of this temporal relationship is unclear, but the data nonetheless document another situation in which a lymphokine activity may be detected *in vivo*.



TEXT-FIGURE 4—Relationship between serum MIF and SGOT in a patient with posttransplant hepatic dysfunction. MIF activity is plotted in terms of a migration index; the lower the value, the greater the activity. (Reprinted from Clin Immunol Immunopathol 3:369–378, 1975, with permission.)

The Use of Antilymphokine Antibodies

As has already been alluded to, there is very little data available on the precise chemical nature of any of the known lymphokines. As stated above, they are usually the products of T cells, although in special circumstances B cells may be also induced to produce lymphokines.[15] In all these situations, multiple lymphokine activities are generated simultaneously. Moreover, these substances are present in small amounts and contaminated by a variety of other products of cellular metabolism. For all these reasons, the isolation and characterization of lymphokines has been extremely difficult and indeed, even MIF, the best studied of these factors, has been characterized only in terms of general physicochemical properties and response to enzyme treatment.

The general availability of antibody to any of the known lymphokines would have obvious importance for their purification and, eventually, for studies on the mechanisms of cell-mediated immunity. However, such antibody production has been difficult to accomplish because of the impurity of the fluids which are the source of such factors. We have found it possible to circumvent this problem by means of a two-stage immunization procedure.[24,25] Although the details of the procedure are beyond the scope of this discussion, it suffices to state that it is possible to produce an antisera in rabbits which, when conjugated to sepharose bead columns,

can selectively remove MIF and MCF activity from activated culture supernatants. Anticontrol activity does not have this property. The antisera so prepared do not have lymphocyte or macrophage cytotoxicity.

The antilymphokine antiserum has proven to be a useful tool in exploring the *in vivo* activity of lymphokines. As previously indicated, lymphokine-containing supernatants can induce mononuclear inflammatory infiltrates when injected into guinea pig skin. This activity has been ascribed to a *skin reactive factor* (SRF) which probably is in reality a mixture of the lymphokines that affect mononuclear cells *in vitro*. The antilymphokine antibody can remove SRF activity from activated supernatants. Of even greater significance is the observation that injection of antilymphokine, but not anticontrol supernatant antibody can profoundly suppress the ability of a previously immunized animal to respond to local antigenic challenge with a cutaneous delayed hypersensitivity reaction. This observation strongly implicates lymphokine involvement in the expression of delayed hypersensitivity.

The Significance of the Lymphokines

We have seen that lymphokines and lymphokine-like factors that are identical or very similar to conventional lymphokines may be produced by a variety of cell types and in a variety of *in vitro* and *in vivo* situations. It is possible to document their participation in the underlying mechanisms of a number of experimental models. Their general significance in the overall biology of the organism, however, remains to be elucidated. I will touch briefly on three aspects of cellular immunity which focus on this point: the cutaneous delayed hypersensitivity reaction itself, infectious immunity, and tumor immunity.

Delayed Hypersensitivity

The evidence for the participation of lymphokines in cutaneous reactions has already been summarized. This includes the detection of chemotactic lymphokines in extracts of such reaction sites, the induction of inflammatory skin reactions by local injection of lymphokines, the suppression of skin reactions by systemic administration of lymphokines, and the suppression of skin reactions by local administration of antilymphokine antibody. Note, however, that this data provides no information on which lymphokine or lymphokines are specifically involved in the skin. Although most textbooks stress the role of MIF in this regard, this is probably based on historic grounds, since for many years MIF was the only lymphokine known. I am aware of no evidence implicating MIF in

cutaneous reactivity. Indeed, our work provides suggestive evidence to the contrary. As already indicated, extracts of skin reactions have chemotactic but not migration inhibitory activity. The failure to detect MIF is probably not due to adsorption to cell surfaces, since such adsorption is very difficult to document convincingly even *in vitro*. It could be due to selective inactivation or destruction of MIF as compared to MCF. However, other evidence points to a role for MCF rather than MIF. As was also discussed, anticoagulants interfere with the expression of cutaneous reaction. *In vitro*, heparin has no effect on the migration inhibition reaction, but we have found that it can inhibit MCF activity.[34] Finally, by appropriate choice of antigen, it is possible to desensitize an animal with respect to either cutaneous reactivity or the macrophage disappearance reaction (MDR). Similarly, one can prevent the appearance of MCF or MIF in such animals. Also, one can achieve either antigen-specific or antigen-nonspecific desensitization with respect to these parameters, depending upon experimental conditions. Correlations of these patterns of inhibition provide evidence that, whereas the MDR appears to be an *in vivo* manifestation of MIF activity, the skin reaction seems to be more dependent upon chemotactic activity.[35]

These results underscore the point that the manifestations of cell-mediated immunity are pleomorphic and the different kinds of reactions may result from different patterns of lymphokine activity in each.

Infectious Immunity

Perhaps the clearest evidence for the role of lymphokines in infectious immunity comes from the work of Mackaness and his co-workers on the role of activated macrophages in resistance to infection by such organisms as *Listeria monocytogenes*. This response was shown to be dependent upon T lymphocytes which secrete lymphokine mediators capable of activating macrophages for this role. This topic, which is beyond the scope of the present discussion, has been extensively reviewed.[36]

Tumor Immunity

A number of investigations in many laboratories (reviewed in Yoshida and Cohen [37]) have provided evidence for several different kinds of lymphokine effects in models involving tumor immunity. Certainly, the role of lymphotoxin in this regard has been exhaustively studied. Like other lymphokines, lymphotoxins, though induced by specific immunologic or mitogenic stimuli, are themselves capable of acting in a nonspecific manner. Although lymphotoxins are thus usually cytotoxic to a variety of both normal and malignant cultured cells, a few reports show

that tumor cells are more susceptible than normal cells to lymphotoxins.

Lymphokines may also interact with tumor cells in an indirect manner by activating inflammatory cells. Just as injection of animals with microorganisms may lead to increased nonspecific resistance to certain other organisms by this mechanism, tumor cells may be killed by activated macrophages. For example, Churchill *et al.* have shown that supernatants from cultures of lymphocytes stimulated by soluble protein antigen activate normal macrophages, either as monolayers or in suspension culture.[38] The responsible lymphokine was called *macrophage activating factor* (MAF). These "activated" macrophages exhibit enhanced cytotoxic capacity against syngeneic Strain 2 hepatoma and MCA-25 sarcoma cells.

Little is known of the mechanism underlying the interaction of the lymphocyte mediator with the surface of the macrophage which results in enhanced cytotoxicity by the activated cells. However, a number of morphologic, biochemical, and functional alterations have been described when macrophages are activated by MAF. These include increased adherence to glass plastic; increased ruffled membrane movement; increased phagocytotic activity; increased glucose oxidation; decrease in levels of lysosomal enzyme, acid phosphatase, cathepsin D, and β-glucuronidase; increase in membrane enzyme adenylate cyclase; increase in incorporation of glucosamine; and enhanced bacteriostasis to *Listeria*. It is interesting that present physicochemical characterization studies cannot distinguish MAF from MIF, although there still remains the possibility that several different factors affecting macrophages exist.

As another possible direct effect of lymphokines in addition to cytotoxic killing of tumor cells, one can consider the effects of lymphokines on the mobility of tumor cells. It has been thought for many years that the mobility of individual tumor cells may be important in local tumor spread and in the establishment of metastasis. It has, however, proved difficult to examine the mobility of tumor cells in *in vitro* systems. Recently, murine lymphoma cells, mastocytoma cells, and various human tumor cells have been shown capable of migration from a capillary tube (reviewed in Churchill *et al.*[38]). This is a simple system which allows a quantitative approach to the examination of the motility of tumor cells *en masse*. The technique is similar to that used in studies on macrophage migration *in vitro* as a model for *in vivo* delayed hypersensitivity.

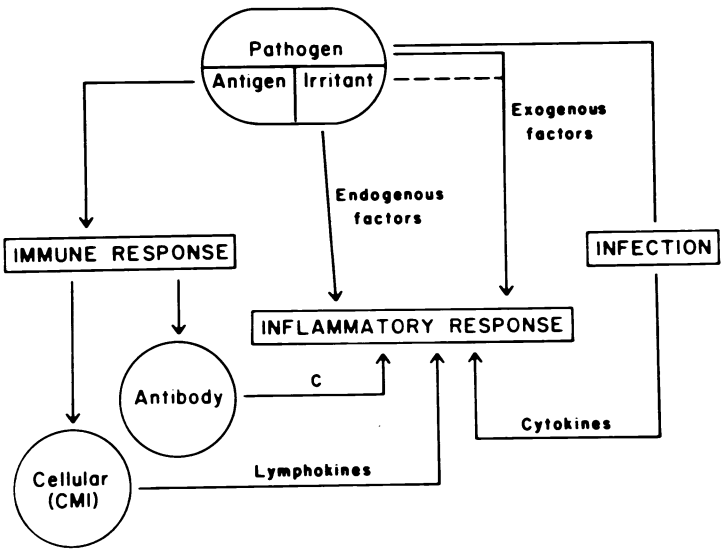
Using this procedure we have shown that lymphokine-containing supernatants can inhibit tumor cell migration *in vitro*. The responsible factor is not separable from MIF. As is the case for the effect of MIF on macrophages, the reaction is not associated with cytotoxicity for the target cell. The initial studies involved the P815 mastocytoma line,[38] but a

number of other tumor cells obtainable in ascitic form all behave in a similar manner. It would be of interest to determine if one could recover this factor from tumor-bearing animals. Also, does this factor change the metastatic capacity of tumors when administered exogenously?

Concluding Remarks

In this presentation, I have discussed the various manifestations of cell-mediated immunity that are attributable to lymphokine-dependent mechanisms, with special attention to the lymphokines that act on inflammatory cells. This represents only a small range of biologic activities. Sensitized lymphocytes can also release substances that affect vascular permeability, and these cells may play a role in collagen synthesis and degradation as well. Thus, they play a pivotal role in various aspects of inflammatory and reparative processes. It is important to emphasize that this role is entirely independent of their ability to make specific antibody. The surprising discovery that nonlymphoid cells can be induced to make lymphokine-like mediators suggests that the lymphokines may be one manifestation of a general biologic phenomenon that is important in host defense and possibly other aspects of homeostasis. In this view, what is unique to the lymphocyte is that it has acquired some specialized means for triggering mediator production not available to other cells. If this notion is correct, then in an evolutionary sense, cell-mediated immunity may be the most primitive expression of immunologic reactivity that has developed out of even more general and nonspecific basic inflammatory responses. The exquisite specificity of the antibody system may represent a further evolution of this process. The fact that the cooperation between T and B cells for antibody synthesis is in part mediated by lymphokine-like molecules provides some tenuous and indirect evidence for this point of view.

Regardless of the means by which this complex system has developed, it is clear that nature has provided us with a remarkable fail-safe system. The safety mechanism is based upon what the aerospace engineers call redundancy. Virtually every property of the lymphokine system I have discussed can be duplicated by some fragment derived from the complement pathway. Also, as we have seen, B cells have the capacity to make lymphokines, although normal circumstances the expression of this capacity appears to be suppressed. Parenthetically, this may explain the ability of certain patients with T-cell immunodeficiencies to mount good antibody responses to T-dependent antigens. Cytokines represent yet another means to the same inflammatory or immunologic end. Some of the multiple pathways are illustrated in Text-figure 5.



TEXT-FIGURE 5—Some of the various pathways leading to the induction of inflammatory responses.

With this remarkable generality of mediator function, it is at first glance difficult to understand the protective capacity of T lymphocytes in various clinical and experimental settings. However, I suspect that mediators in the local environment near a lesion, such as those produced by the infected cells, may not be very important in terms of initiating a protective inflammatory response. After all, one must mobilize cells from the marrow and arrange for their migration through vessels as well as for their attraction, immobilization, and activation. The lymphocyte is a perfect cell for these activities since it travels with the inflammatory crowd. It is a member of a population capable of rapid expansion, and it is capable of delivering a "shot" of mediators when and where it is most needed, at some proximity to the target cells for those mediators.

Another problem that awaits resolution relates to the control mechanisms involved in lymphokine-dependent reactions. As we have seen, one of the lymphokines is a mitogenic factor, and it is known that this mediator can act on both T and B cells. Since mitogenic activation is a sufficient trigger for lymphokine production, we should have an endless cycle of production, release, and stimulation until our total mass consists of lymphokines. While this might be an attractive prospect for immunologists looking for large amounts of mediators for purification and characterization studies, it is hardly a biologically useful end stage. Obviously, regulatory mechanisms must exist. The inhibition of B cell–derived MIF

by MIFIF may provide a clue to the nature of these regulatory mechanisms. Much future effort will have to be devoted to this issue.

Finally, it should be apparent that most of the discoveries in this field have occurred in spite of the available methodology, rather than because of it. It is exceedingly difficult to study phenomena whose detection requires complex, crude, time-consuming, and relatively unquantitative biologic assays. For example, seemingly simple questions relating to the interaction of mediators with putative surface receptors on target cells have proven impossible to answer. This has been the impetus for the introduction of more biochemically and physicochemically based assays in many laboratories throughout the world and has also led us and others to devise techniques for raising antisera to various lymphokines. These modern approaches hold promise for the development of radioimmunoassay and single cell assays which will be indispensable for further progress in these areas. Hopefully, this will enable lymphokine biology to rest on as firm an immunochemical basis as does the antibody system.

References

1. McCluskey RT, Leber PD: Cell-mediated reactions in vivo. Mechanisms of Cell-Mediated Immunity. Edited by RT McCluskey, S Cohen. New York, John Wiley & Sons, Inc., 1974, p 1
2. Cohen S, McCluskey RT, Benacerraf B: Studies on the specificity of the cellular infiltrate of delayed hypersensitivity reactions. J Immunol 98:269–273, 1967
3. Cohen S, Benacerraf B, McCluskey RT, Ovary Z: Effect of anticoagulants on delayed hypersensitivity reactions. J Immunol 98:351–358, 1967
4. Nelson DS: The effects of anticoagulants and other drugs on cellular and cutaneous reactions to antigen in guinea-pigs with delayed-type hypersensitivity. Immunology 9:219–234, 1965
5. Dvorak HF, Mihm MC Jr, Dvorak AM, Johnson RA, Manseau EJ, Morgan E, Colvin RB: Morphology of delayed type hypersensitivity reactions in man. I. Quantitative description of the inflammatory response. Lab Invest 31:111–130, 1974
6. Cohen S: Cell-mediated immunity and the inflammatory system. Human Pathol 7:249–264, 1976
7. Remold HG, David JR: Migration inhibition factor and other mediators in cell-mediated immunity.[1] p 25
8. Cohen S, Ward PA, Bigazzi PE: Cell cooperation in cell-mediated immunity.[1] p 331
9. Ward PA, Remold HG, David JR: The production by antigen-stimulated lymphocytes of a leukotactic factor distinct from migration inhibitory factor. Cell Immunol 1:162–174, 1970
10. Rocklin RE: Role of monosaccharides in the interaction of two lymphocyte mediators with their target cells. J Immunol 116:816–820, 1976
11. Cohen S, Ward PA: In vitro and in vivo activity of a lymphocyte and immune complex-dependent chemotactic factor for eosinophils. J Exp Med 133:133–146, 1971
12. Torisu M, Yoshida T, Ward PA, Cohen S: Lymphocyte-derived eosinophil chemotactic factor. II. Studies on the mechanism of activation of the precursor substance by immune complexes. J Immunol 111:1450–1458, 1973

13. Leber PD, Milgrom M, Cohen S: Eosinophils in delayed hypersensitivity skin reaction sites. Immunol Commun 2:615–620, 1973
14. Cohen S, Rose NR, Brown RC: The appearance of eosiniphils during the development of experimental autoimmune thyroiditis in the guinea pig. Clin Immunol Immunopathol 2:256–265, 1974
15. Yoshida T, Sonozaki H, Cohen S: The production of migration inhibition factor by B and T cells of the guinea pig. J Exp Med 138:784–797, 1973
16. Rocklin RE, MacDermott RP, Chess L, Schlossman SF, David JR: Studies on mediator production by highly purified human T and B lymphocytes. J Exp Med 140:1303–1316, 1974
17. Mackler BF, Altman LC, Rosenstreich DL, Oppenheim JJ: Induction of lymphokine production by EAC and of blastogenesis by soluble mitogens during human B-cell activation. Nature 249:834–837, 1974
18. Wahl SM, Iverson GM, Oppenheim JJ: Induction of guinea pig B-cell lymphokine synthesis by mitogenic and nonmitogenic signals to Fc, Ig, and C3 receptors. J Exp Med 140:1631–1645, 1974
19. Bloom BR, Stoner G, Gaffney J, Shevach E, and Green I: Production of migration inhibitory factor and lymphotoxin by non-T cells. Eur J Immunol 5:218–220, 1975
20. Epstein LB, Kreth HW, Herzenberg LA: Fluorescence activated cell sorting of human T and B lymphocytes. II. Identification of the cell type responsible for interferon production and cell proliferation in response to mitogens. Cell Immunol 12:407–421, 1974
21. Yoshida T, Kuratsuji T, Takada A, Takada Y, Minowada J, Cohen S: Lymphokine-like factors produced by human lymphoid cell lines with B or T cell surface markers. J Immunol 117:548–554, 1976
22. Bloom BR, Shevach E: Requirement for T cells in the production of migration inhibitory factor. J Exp Med 142:1306–1311, 1975
23. Hammond ME, Roblin RO, Dvorak AM, Selvaggio SS, Black PH, Dvorak HF: MIF-like activity in simian virus 40–transformed 3T3 fibroblast cultures. Science 185:955–957, 1974
24. Yoshida T, Bigazzi PE, Cohen S: The production of anti–guinea pig lymphokine antibody. J Immunol 114:688–691, 1975
25. Kuratsuji T, Yoshida T, Cohen S: Anti-lymphokine antibody. II. Specificity of biological activity. J Immunol 117:1985–1991, 1976
26. Yoshida T, Bigazzi PE, Cohen S: Biologic and antigenic similarity of virus-induced migration inhibition factor to conventional, lymphocyte-derived migration inhibition factor. Proc Natl Acad Sci USA 72:1641–1644, 1975
27. Sonozaki H, Cohen S: The macrophage disappearance reaction: Mediation by a soluble lymphocyte-derived factor. Cell Immunol 2:341–352, 1971
28. Cohen S, Ward PA, Yoshida T, Burek CL: Biologic activity of extracts of delayed hypersensitivity skin reaction sites. Cell Immunol 9:363–376, 1973
29. Yoshida T, Benacerraf B, McCluskey RT, Vassalli P: The effect of intravenous antigen on circulating monocytes in animals with delayed hypersensitivity. J Immunol 102:804–811, 1969
30. Yoshida T, Cohen S: Lymphokine activity in vivo in relation to circulating monocyte levels and delayed skin reactivity. J Immunol 112:1540–1547, 1974
31. Cohen S, Fisher B, Yoshida T, Bettigole RE: Serum migration inhibitory activity in patients with lymphoproliferative diseases. N Engl J Med 290:882–886, 1974
32. Yoshida T, Edelson R, Cohen S, Green I: Migration inhibitory activity in serum and cell supernatants in patients with Sezary syndrome. J Immunol 114:915–918, 1975
33. Torisu M, Yoshida T, Cohen S: Serum migration inhibitory activity in patients with post-transplantation hepatic dysfunction. Clin Immunol Immunopathol 3:369–376, 1975

34. Cohen S, Yoshida T: Unpublished data
35. Sonozaki H, Papermaster V, Yoshida T, Cohen S: Desensitization: Effects on cutaneous and peritoneal manifestations of delayed hypersensitivity in relation to lymphokine production. J Immunol 115:1657–1661, 1975
36. North RJ: Cell-mediated immunity and the response to infection.[1] p 185
37. Yoshida T, Cohen S: Lymphokines in tumor immunity. Mechanisms of Tumor Immunity. Edited by I Green, S Cohen, RT McCluskey. New York, John Wiley & Sons, Inc., 1977, p 87
38. Churchill WH Jr, Piessens WF, Sulis CA, David JR: Macrophages activated as suspension cultures with lymphocyte mediators devoid of antigen become cytotoxic for tumor cells. J Immunol 115:781–786, 1975
39. Cohen MC, Zeschke R, Bigazzi PE, Yoshida T, Cohen S: Mastocytoma cell migration in vitro: Inhibition by MIF-containing supernatants. J Immunol 114:1641–1643, 1975

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