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Biologic and Antigenic Similarity of Virus-Induced Migration Inhibition Factor to Conventional, Lymphocyte-Derived Migration Inhibition Factor

(lymphokines/cellular immunity/delayed hypersensitivity/inflammatory mediators/cytokines)

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ABSTRACT **Macrophage migration inhibition factor (MIF) is one of a class of lymphocyte-derived mediator substances (lymphokines) which plays a role in the mechanism of cellular immunity. A variety of other soluble factors produced by non-lymphoid cells have been shown to have effects on macrophage mobility similar to that of MIF. In the present study we demonstrate that one such factor, (MIF_V) derived from simian virus 40-infected kidney cells in culture, has several other properties in common with lymphocyte-derived MIF (MIF_L). MIF_V can be adsorbed on Sepharose bead columns conjugated with an antiserum prepared against MIF_L, demonstrating at least some antigenic similarity. Moreover, MIF_V can substitute for MIF_L in an *in vivo* system involving the suppression of cutaneous manifestations of cellular immunity by intravenous injection of the lymphokine. These observations, taken in conjunction with the similarity of the *in vitro* effect of MIF_V and MIF_L, and their similar chromatographic behavior, suggest that MIF_V and MIF_L may be identical molecular species.**

A number of soluble, non-antibody mediator substances (lymphokines) may be produced by lymphocytes under various experimental conditions. The classic situation for lymphokine production involves the stimulation of sensitized lymphocytes by specific antigen. In addition, it has been shown that mitogens can induce the production of similar factors by nonsensitized lymphocytes (1, 2). On the basis of much indirect evidence, it was assumed that thymus-derived lymphocytes (T cells) were the source of lymphokines. It has been recently demonstrated by Yoshida *et al.* (3) and subsequently confirmed by Rocklin *et al.* (4) that under appropriate *in vitro* conditions, bone marrow-derived lymphocytes (B lymphocytes) as well as T lymphocytes can produce the lymphokine migration inhibition factor (MIF). In addition, the prior studies (3) had demonstrated that the soluble mediator of the macrophage disappearance reaction, an *in vivo* manifestation of cellular immunity, could be produced by B cells as well as T cells.

Substances with lymphokine-like activity are ubiquitous in nature. Thus, a variety of cell types other than lymphocytes can release MIF or MIF-like factors into tissue culture (5, 6). Similar factors may appear in the serum of either immunized animals (7, 8) or patients with various lymphoproliferative disorders (9). Finally, the infection of a variety of cells by viruses such as mumps and Newcastle disease virus *in vitro* (10, 11) or *in vivo* (12), and simian virus (SV40) *in vitro* (13, 14) leads to the production of lymphokine-like substances. Thus, the production of these factors seems to represent a general biologic phenomenon.

Mediator substances with similar biologic activity, though obtained from diverse cellular sources, may have strikingly similar physicochemical properties. However, few investigators feel comfortable in labeling them in such a manner as to imply even functional identity. This is most frequently the case in studies of macrophage migration inhibitory factors. Accordingly, we felt it to be of importance to attempt to clarify the relationship between MIF's from different sources.

Recently, we have shown that administration of exogenous MIF-rich lymphocyte culture supernatants into guinea pigs actively immunized to unrelated antigens could suppress delayed skin reactions in those recipients (8). In an unrelated study, we have demonstrated that it is possible to prepare antisera against culture supernatants obtained from antigen-activated cultures of sensitized lymphocytes. We have shown that those antisera can suppress various lymphokine activities *in vitro* and *in vivo* (15). These two systems, one involving suppressive effects of excess, exogenous lymphokine, and the other involving suppression by specific anti-lymphokine antisera, seemed ideally suited to a study of the relationship of non-lymphocyte-derived MIF to conventional MIF. In the present report, we so characterize a macrophage migration inhibition factor obtained from the infection of monkey kidney cells with SV40 virus.

METHODS AND MATERIALS

Lymphocyte Culture Supernatants. Supernatants with MIF activity were obtained as described previously (8, 15). Briefly, Hartley albino guinea pigs (350–400 g) were immunized by foot pad injections of 100 μg of dinitrophenylated bovine serum albumin in Freund's complete adjuvant. Lymphocytes were obtained 8–10 days later, and 1.5×10^7/ml of the cells suspended in RPMI 1640 (ABS, Buffalo, N.Y.), supplemented with 50 μg/ml of streptomycin and 50 units/ml of penicillin, were cultured with or without the presence of specific antigen (50 μg/ml). Culture supernatants were obtained by centrifugation at $1850 \times g$ for 30 min. Control supernatants, obtained from cells cultured without antigen, were reconstituted with antigen prior to use. MIF obtained from these cell prepa-

Abbreviations: MIF, migration inhibition factor; MIF_V, migration inhibition factor derived from simian virus 40 infected kidney cells in culture; MIF_L, migration inhibition factor derived from lymphocytes; AGMK, African green monkey kidney; T cell or T lymphocyte, thymus-derived lymphocyte; B cell or B lymphocyte, bone marrow-derived lymphocyte; SV40, simian virus 40; Lf, limit of flocculation.

TABLE 1. *Suppression of delayed skin reactions by systemic injections of MIF-rich supernatants*

Injected material	Skin reaction diameters (24 hr) Before injection	After injection	% Reduction†
Lymphocyte-derived			
$Control_L$*	14.5 ± 1.5	12.8 ± 0.7	
MIF_L	15.3 ± 2.0	5.2 ± 1.8	82.9
SV40-AGMK			
$Control_V$*	15.5 ± 0.6	13.5 ± 1.1	
MIF_V	16.0 ± 1.7	7.1 ± 1.5	79.2
No injection	14.5 ± 0.9	13.8 ± 2.1	

* Each control consisted of supernatant from the appropriate unstimulated culture, reconstituted with antigen or virus prior to use.

† % Reduction = (difference in area between skin reactions in experimental and control supernatants area in control) × 100 = $(diameter^2_{cont} - diameter^2_{exp})/diameter^2_{cont} \times 100$.

rations will be called MIF_L to distinguish it from the virus-induced factor described below. Similarly, the antigen-reconstituted control will be called $Control_L$.

Virus-Infected Kidney Cell Cultures. Virus-induced MIF (MIF_V) was obtained by inoculating African green monkey kidney (AGMK) cells (GIBCO, Grand Island, N.Y.) with SV40 virus and harvesting supernatants 24 hr later as previously described (13). Complete monolayers of these cells in 75 cm^2 flasks were inoculated with 0.5 ml of undiluted SV40 virus which represents a cytolytic dose [titer: $10^{5.8}$TCID (tissue culture infectious dose)$_{50}$/0.1 ml]. The cultures were incubated at room temperature for 20 min. After this period, 25 ml of Eagle's minimal essential medium supplemented with 2% fetal calf serum were added and the flasks incubated at 37°. Control flasks were incubated initially with 0.5 ml of medium and then treated in a similar way. After 24 hr of incubation, the supernatants from the infected and the control flasks were removed, UV-irradiated and centrifuged at 1850 × *g* for 30 min. Control supernatants ($Control_V$) were reconstituted with UV-irradiated virus.

Sephadex G-100 Gel Filtration. Lyophilized supernatants were dissolved in cold distilled water to achieve a 30-fold concentration of the initial supernatant fluid. Any resulting insoluble material was removed by centrifugation. Such removal had been confirmed not to reduce significantly the MIF activity in the supernatant. Three milliliters of the concentrated supernatant was applied on Sephadex G-100 columns (2.5 × 50 cm) and eluted with 0.1 M phosphate buffered saline. Eluates were collected as 3 ml aliquots. MIF-containing fractions were pooled, as were the corresponding fractions obtained from control supernatants. These pools were obtained from fractions containing material in the molecular weight range of 45,000–67,000.

Anti-Lymphokine Antiserum. The preparation used in these studies was one which had been previously described and characterized (15). It was produced by a two-stage procedure. In brief, MIF_L and control supernatants were obtained from guinea pig lymphocyte cultures. They were fractionated on Sephadex G-100. Fractions of control supernatants corresponding to active fractions from the MIF-rich material were used to immunize rabbits. The anti-control antibody so obtained was conjugated to agarose beads. The MIF-rich Sephadex fractions were passed through this immunoadsorbent column to remove materials corresponding to those found in control supernatants and which could react with the anti-control antibody. The eluted, MIF-rich fraction was concentrated and used to immunize a second group of rabbits. The anti-lymphokine antibody so obtained was found capable of suppressing the activity of MIF_L, macrophage chemotactic factor, and skin reactive factor. In that study (15) it could also suppress delayed hypersensitivity reactions *in vivo* in actively immunized guinea pigs.

Preparation of Immunoadsorbents. The anti-lymphokine and anti-control antisera described above were bound to agarose beads (Sepharose 2B) activated by cyanogen bromide as described previously (15). Activated agarose beads (250 ml packed volume) were reacted with 50 ml of either antiserum at 4° for 18–24 hr. They were washed with 10 volumes of phosphate-buffered saline, 10 volumes of acetic acid, 2 volumes of 2% fetal calf serum, and 10 volumes of phosphate-buffered saline in that order. The antiserum-conjugated beads were used as immunoadsorbents within 24 hr of such preparation.

Migration Inhibition Assay. For experiments to determine the effect of anti-lymphokine sera on MIF_V and MIF_L activity, macrophage migration inhibition assays were performed as we have described previously (8, 15). The area of migration in the medium containing test samples was compared with that in the medium containing control samples. The migration index was calculated as described previously (16):

$$MI = 100 \times \frac{\text{migration area in test medium}}{\text{migration area in control medium}}.$$

RESULTS

Immunosuppression with exogenous MIF_V and MIF_L

Guinea pigs were immunized with diphtheria toxoid–antitoxin complexes containing 50 Lf (limit of flocculation) of the toxoid in Freund's incomplete adjuvant. Two weeks later, they were skin-tested in one flank by 5 Lf of toxoid in phosphate-buffered saline. Reactions were examined at intervals to monitor the evolution of the typical delayed hypersensitivity cutaneous reactions. At 24 hr, groups of immunized guinea pigs (six animals per group) received an intravenous injection of Sephadex-fractionated MIF_L (MW 45,000–67,000) or Sephadex fractionated MIF_V (MW 45,000–67,000) or the corresponding fractions of appropriate control fluids. Each animal represented a single intravenous injection of 1 ml of fluid which was 10-fold concentrated in MIF activity with respect to the initial supernatants. Immediately afterward, each animal was skin-tested with 5 Lf of toxoid in the other flank, and reactions were observed over the next 24 hr. This procedure, in which each animal serves as its own control, minimizes fluctuations in reaction intensity due to variations in responsiveness in different guinea pigs. The results are shown in Table 1. Each measurement represents diameter of induration and erythema. Both MIF_L and MIF_V were capable of significant diminution of skin reactivity. The percent reduction, as calculated from the difference in area between experimental and control sites, is 82.9% when MIF_L is used

and 79.2% when MIF_V is used. The control fluids, which were reconstituted with either antigen or virus, as described in *Methods and Materials*, were without significant effect.

Effect of anti-lymphokine serum on MIF_V and MIF_L

Immunoadsorbent columns containing either anti-lymphokine or anti-control antibody were prepared as described above. As already indicated, these antisera were prepared against supernatant fluids obtained from antigen-activated and control lymphocyte cultures. Five milliliters each of MIF_V- or MIF_L-containing supernatants, or their respective controls, were passed through the immunoadsorbent columns packed with 30 ml of either anti-control conjugated or anti-lymphokine conjugated agarose beads. After incubation at room temperature for 60 min, each column was eluted by 50 ml of RPMI 1640. The eluates were lyophilized and reconstituted to the original volume (5 ml each), and then dialyzed against RPMI 1640. These preparations were supplemented with 20% normal guinea pig serum and tested for MIF activity. The results are shown in Table 2. Adsorption with Sepharose beads conjugated with anti-control antibody had no effect on the activity of either the MIF_L or the MIF_V-containing preparations. The migration indices of 63.2 and 69.7, respectively, were unchanged from that of unadsorbed supernatants. (The original unadsorbed MIF_L and MIF_V at the same concentration had migration indices of 63.5 ± 4.6 and 70.8 ± 1.8.) In contrast, adsorption with the antilymphokine columns removed virtually all activity from both the MIF_V and MIF_L supernatants. These migration indices all represent grand averages based upon four complete experiments.

It should be noted that the calculations of migration indices in these experiments are always made relative to the migration of macrophages in the appropriate control fluid which had been passed through the appropriate Sepharose column. In any case, passage of any control fluid through any column had no effect on its ability to support macrophage migration.

TABLE 2. *Effect of anti-lymphokine serum on migration inhibitory activity in antigen-stimulated or SV40-infected cell culture supernatants*

Culture supernatants	Immunoadsorbent	Migration index*
$Control_L$	Anti-control	(100)
MIF_L	Anti-control	63.2 ± 5.7
$Control_V$	Anti-control	(100)
MIF_V	Anti-control	69.7 ± 4.3
$Control_L$	Anti-lymphokine	(100)
MIF_L	Anti-lymphokine	95.8 ± 4.6
$Control_V$	Anti-lymphokine	(100)
MIF_V	Anti-lymphokine	97.7 ± 5.1

* The figures represent grand averages (mean value calculated from the averages for each experiment) based upon the results of four complete experiments. In each experiment, all permutations involving MIF_L, MIF_V, anti-control and anti-lymphokine sera were performed.

DISCUSSION

Viral infection of a variety of cells *in vitro* and *in vivo* leads to the production of substances with lymphokine-like activity (10–14). Such substances include macrophage migration inhibition factors, neutrophil and macrophage chemotactic factors, and skin reactive factors. Recently, we have reported (13) that SV40 virus can induce the production of an MIF-like factor by AGMK cells, in the absence of the concomitant appearance of other lymphokine-like activities. Sephadex chromatography of active supernatants revealed the MIF activity to be in fractions corresponding to a molecular weight range of 45,000–67,000 and in another fraction corresponding to an approximate molecular weight of 12,000. This distribution corresponds precisely to that of conventional, lymphocyte-derived MIF_L (17).

The exact relationship of the virus-derived factor (MIF_V) to MIF_L is difficult to determine because of the lack of precise biochemical characterization data for either. The issue is of importance, since the wide range of experimental settings in which lymphokine and lymphokine-like activity can be detected suggests that these factors may be ubiquitous in nature, and that their biologic role is not confined to the immunologic system. These factors, for the most part, exert their effect on various inflammatory cells. If these agents from different cellular sources can be shown to be chemically as well as functionally identical, this identity will provide a unifying framework for the various aspects of host defense which involve the inflammatory system.

In the present study we have compared MIF_L and MIF_V in two ways. First, we have shown that MIF_V is as capable as conventional MIF_L in suppressing cutaneous manifestations of delayed hypersensitivity when injected intravenously. The mechanism of this immunosuppressive effect is not clear, but it is thought to involve activation and immobilization of macrophages systemically, so that they are pre-empted from participating in the cutaneous reaction (8). In any case, this experimental procedure demonstrates that MIF_V shares, *in vivo* as well as *in vitro*, properties with MIF_L.

The second comparison of MIF_V and MIF_L made here is the demonstration that both may be removed from supernatants by passage through an immunoadsorbent column containing antibody raised against MIF_L. This antigenic cross-reactivity between factors obtained from widely different sources (antigen-activated lymphocytes versus virus-infected kidney cells) suggests at least partial chemical homology.

The present study provides one possible explanation for the intriguing observation that many viral infections lead to a depression of the expression of preexisting delayed hypersensitivity (18, 19). In one of these studies (18), it was shown that sensitivity could be transferred from infected animals with depressed skin reactivity to normal animals, demonstrating that there was no defect in the function of specifically sensitized lymphocytes. Since exogenous MIF can suppress skin reactivity, it seems likely that high circulating levels of endogenous virus-induced MIF could do so as well. The demonstration that mumps virus can induce MIF *in vivo* as well as *in vitro* (11, 12) is evidence for this mechanism of suppression. However, further work is required correlating cutaneous reactivity, serum MIF, and *in vitro* manifestations of cellular immunity during the course of viral infections.

The results reported here lend further support to the view that various substances with a particular lymphokine-like activity may have chemical as well as functional properties in common, even though derived from diverse sources. It is tempting to postulate that the ability to produce these agents represents one of the most primitive self-defense mechanisms

and that cellular immunity, to the extent that it is dependent upon lymphokine production, represents a subsequent evolution of that system.

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