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Migration Inhibitory Factor and Macrophage Bactericidal Function

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A homogeneous population of immunologically active lymphocytes was obtained from peritoneal exudates of guinea pigs with delayed hypersensitivity to bovine gamma globulin (BGG). The lymphocytes were cultured with and without BGG for 24 hr, and cell-free supernatant fluids were then assayed simultaneously for their ability to influence two in vitro parameters of macrophage function: migration from capillary tubes and bactericidal capacity. In four consecutive experiments, supernatants from antigenically stimulated lymphocytes exhibited substantial migration-inhibitory-factor activity without enhancing the ability of macrophages to kill *Listeria monocytogenes*. Lymphocyte lysates were inactive in both assays. Possible mechanisms of lymphocyte-macrophage interactions are discussed.

Successful host defense against infection with certain intracellular microorganisms appears to require the enhancement of macrophage (MP) microbiocidal activity. In vivo studies by Mackaness and his colleagues (6, 7) have elucidated the immunological events responsible for such macrophage activation. Recently we described (13, 14) an in vitro model of cellular immunity which confirms these findings. In both systems, when committed lymphocytes are stimulated by specific antigen, they are able to greatly enhance the ability of normal MP to kill antigenically unrelated bacteria. However, the mechanism of the lymphocyte-MP interaction remains uncertain. Speculation has centered on the possibility that the antigenically stimulated sensitized lymphocyte produces a soluble mediator, such as migration inhibitory factor (MIF), which in turn activates MP bactericidal mechanisms. In our system (14), simultaneous experiments showed that sensitized lymphocytes were able to inhibit MP migration under the same conditions which allowed them to enhance MP bactericidal capacity; moreover, in both MIF and bactericidal assays, peritoneal exudate lymphocytes were functionally superior to lymph node lymphocytes, and in both assays the magnitude of the MP response was directly related to the number of lymphocytes present. The present studies were carried out to test the hypothesis that MIF is directly responsible for the activation of MP bactericidal mechanisms.

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MATERIALS AND METHODS

Techniques previously described in detail (13, 14) will only be summarized. Strain 2 guinea pigs were immunized with 0.125 mg of bovine globulin (BGG) in complete Freund's adjuvant. Four to six weeks later, peritoneal exudates were induced with mineral oil, and the peritoneal exudate cells (PEC) from six to eight animals were pooled. In experiments 1 and 2 (Table 1), a sample of PEC was assayed in the bactericidal system: 4×10^6 PEC were incubated for 24 hr in 35-mm petri dishes in 2 ml of enriched minimal essential media (EMEM) containing 20% heated normal guinea pig serum in the presence or absence of 10 μg of BGG per ml. After being washed, the MP monolayers were infected with 5×10^7 *Listeria monocytogenes* cells. After 30 min of incubation, the monolayers were washed and reincubated in EMEM containing 1.2 μg of penicillin per ml; 3.5 hr later, three or four monolayers from each group were washed and lysed with distilled water. Serial dilutions were made to quantitate viable intracellular bacteria. As in our previous studies (13, 14), cell numbers and phagocytic activity were indistinguishable in the PEC incubated with and without antigen; thus differences in viable bacteria reflect differences in MP killing.

In all experiments, lymphocytes were separated by incubating PEC on viscose rayon columns for 1 hr. The cells eluted from these columns with warm EMEM were >95% viable lymphocytes, which were washed, adjusted to a concentration of 5×10^6/ml, and incubated in serum-free EMEM containing 100 units of penicillin/ml with or without 10 μg of BGG per ml. After 24 hr, supernatant fluids were separated by centrifugation, and BGG (10 μg/ml) was added back to the supernatants which had none. Supernatant fluids were then added to freshly harvested normal PEC and incubated with 20% normal serum for 24 hr

TABLE 1. *Effect of supernatant fluids and lysates of BGG immune lymphocytes cultured with and without antigen on bactericidal capacity and migration of normal macrophages*

Expt.	Donor exudates		Lymphocyte supernatants		Lymphocyte lysates	
	Immune + Ag	Immune − Ag	Immune + Ag	Immune − Ag[a]	Immune + Ag	Immune − Ag
1. Bactericidal	165 (146–186)[c]	1,636[b] (1,440–2,000)	1,072 (1,000–1,140)	902 (680–1,080)	—	—
MIF	—	—	146.3 ± 13.1[d]	401.8 ± 21.3[b]	—	—
2. Bactericidal	177 (134–230)	900[b] (798–1,020)	1,055 (982–1,102)	1,126 (1,024–1,266)	—	—
MIF	—	—	65.3 ± 4.1	198.3 ± 6.8[b]	—	—
3. Bactericidal	—	—	2,120 (1,848–2,382)	1,731 (1,476–2,054)	1,857 (1,706–2,014)	1,622 (1,414–1,806)
MIF	—	—	216.3 ± 45.7	484.3 ± 23.1[b]	360.3 ± 18.5	355 ± 19.5
4. Bactericidal	—	—	454 (406–500)	387 (330–442)	621 (596–746)[a]	711 (542–1,006)[a]
MIF	—	—	109.8 ± 11.3	298 ± 6.7[b]	313.5 ± 15[a]	266 ± 32.6[a]

[a] Antigen added to material prior to assay.

[b] $P < 0.01$ t test; all other comparisons not significantly different.

[c] Geometric mean (range of viable intracellular listeria $\times 10^{-3}$/plate at 4 hr. Three or four duplicate monolayers in each group.

[d] Mean ± standard error in planimeter units. Four capillary tubes, in two chambers, in each group.

for the bactericidal assay as outlined above. Supernatants were simultaneously assayed for MIF activity with the same normal PEC by standard techniques (1).

In experiments 3 and 4 (Table 1), the lymphocytes which had been incubated for 24 hr to make supernatant fluids were resuspended in fresh EMEM at a concentration of 5×10^6 cells/ml and lysed by 10 cycles of freeze-thawing. The lymphocyte lysates were tested in both MIF and bactericidal assays simultaneously with the supernatant fluids.

RESULTS AND DISCUSSION

Table 1 summarizes four consecutive experiments. In experiments 1 and 2, the PEC from BGG-immune animals ("Donor exudates") clearly responded to incubation with BGG by marked enhancement of MP listericidal capacity ($P < 0.001$, t test). This is the basic reaction which we previously explored in detail (13, 14). In all four experiments, lymphocytes purified from immune PEC were incubated with and without BGG. Supernatants fluids from antigenically stimulated lymphocytes markedly inhibited the migration of normal MP: areas of migration were decreased by 55 to 67% of MP incubated with supernatants from unstimulated lymphocytes ($P < 0.01$). This was not a nonspecific effect of BGG, for, as noted above, this antigen was added to the control supernatants prior to assay. These same supernatants were simultaneously assayed for their effect on MP bactericidal capacity. It is clear from the table that MIF-rich supernatants had no effect on MP listericidal activity. In experiments 3 and 4, lysates of antigenically stimulated and unstimulated lymphocytes were tested in both assays but had no demonstrable effect.

There is no doubt that supernatants from antigenically stimulated lymphocytes can affect MP functions in addition to migration; the question is whether such materials cause enhancement of bacteridical activity per se. Mooney and Waksman (9) demonstrated in a rabbit system that such supernatants produced increased MP ameboid activity after 24 hr and increased adherance at 48 hr. Nathan et al. (10) showed that purified guinea pig MIF produced increased MP adherance, phagocytosis, motility, and hexose monophosphate shunt activity after 72 hr. Neither of these studies report MP bactericidal activity, and both require more than the 24 hr which is needed for enhancement of MP bacteridical activity in our system (13). Patterson and Youmans (11) reported that if lymphocytes from tuberculin-sensitive mice were incubated with tubercle bacilli, they produced a soluble factor which suppressed the multiplication of tubercle bacilli in normal MP after 3 to 6 days. Godal et al. (2) found that supernatants from rabbit mixed leukocyte cultures suppressed the multiplication of mycobacteria in normal peripheral blood monocytes after 10 to 17 days. Neither of these studies evaluated the supernatants for MIF activity and both required prolonged periods of incubation. Recently, however, Krahenbuhl and Remington (5) described a guinea pig system in which they conferred resistance to listeria infection on normal MP with spleen cells from

toxoplasma-infected animals in the presence of toxoplasma antigen. This study also reports an experiment in which supernatants from cultures of normal MP, immune spleen cells, and toxoplasma antigen enabled normal MP to resist listeria infection after 72 hr. They also state that, in two different experiments, similar supernatants inhibited the migration of normal MP by 33 and 34%. In the four experiments reported in the present study, however, supernatants which inhibited MP migration by 55 to 67% did not enhance MP bactericidal activity when assayed simultaneously.

The disparity between the studies (2, 5, 11) which suggest that lymphokines may enhance MP bactericidal activity and the present experiments may reflect differences in experimental conditions. Indeed, we have not attempted to duplicate the conditions reported by these other investigators. Rather, we have evaluated the role of lymphocyte supernatants under conditions identical to our previous studies of whole peritoneal exudates (13) and purified lymphocytes (14). In these earlier studies, we presented indirect evidence that MIF activity and bactericidal enhancing activity were inseparable. When antigenically stimulated immune lymphocytes were added to normal macrophages, MP migration was inhibited and bactericidal activity was enhanced in parallel. The present experiments present direct evidence of a dissociation between these phenomena for the first time. Despite possessing a high degree of MIF activity, supernatants from antigenically stimulated immune lymphocytes had no effect on MP bactericidal capacity.

Clearly, the absence of proof does not constitute proof of absence, and the present study of a single in vitro model does not disprove the popular hypothesis that MIF is a mediator of enhanced resistance to infection with intracellular parasites. Further investigation will be required to evaluate the biological validity of the various in vitro models which have been reported. Nevertheless, the dissociation of migration inhibition and bactericidal enhancement in a well-defined system may serve to stimulate critical evaluations of this question.

What are the other possible mechanisms of lymphocyte-macrophage interaction? Cytophilic antibody remains a possibility, though in vivo (8) and in vitro (14) studies with immune serum argue against its role. Another possibility is that the antigenically stimulated sensitized lymphocyte retains a substance within its cytoplasm or tightly bound to its surface which activates MP bactericidal machinery. In the present experiments, we could not demonstrate such a material in crude lymphocyte lysates. However, others have noted that ribonucleic acid extracts from sensitized lymphocytes could induce MIF production by normal lymphocytes in the presence of antigen (4). An alternate way in which the stimulated lymphocyte could influence the MP is through elaboration of a soluble mediator which is more labile than MIF and hence not demonstrable in this system. Finally, direct lymphocyte-MP membrane contact may be required, as is the case in one form of the cytotoxicity reaction (3). Such intimate lymphocyte-MP interaction is perhaps suggested by our inability to separate these cell types by repeatedly washing monolayers (14), and by the morphological observations of Salvin et al. (12).

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