PRODUCTION OF FACTORS REGULATING MACROPHAGE MOBILITY IN THE EARLY PERIOD OF MIXED LYMPHOCYTE CULTURE

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UDC 612.122.94.017.1:612.112.2

On contact with specific antigen immune T lymphocytes are activated and produce biologically highly active substances known as lymphokines, including macrophage migration inhibition factor (MIF). MIF has a local action on macrophages, promoting their activation and accumulation in a focus of reaction of delayed type of hypersensitivity [5], and also a systemic action, on the number of monocytes circulating in the blood stream [12]. Besides MIF, T-lymphocytes can also synthesize a macrophage migration stimulation factor (MSF), which is not identical with MIF [3, 7] and which can neutralize its activity [8]. It has been suggested that MIF production is under the control of T suppressors which produce MSF [8]. A study of the macrophage migration inhibition reaction in a system of transplantation or antitumor immunity has frequently revealed stimulation of macrophage migration [4, 6], the appearance of which correlated with weakening of these forms of immunity. It was shown previously that on the 2nd-3rd day in mixed lymphocyte culture (MLC) in a H-2 system a sharp peak of MIF activity is observed [1, 10].

The object of the present investigation was to study the rhythm of MIF and MSF production in MLC.

EXPERIMENTAL METHOD

Spleen cells of individual C57BL/6 (abbreviated to B6) (H-2^b) mice, mixed with irradiated (^{60}Co, 1500 rads) spleen cells from B10 × D2 (H-2^d) mice (allogeneic MLC) or spleen cells of B6 mice (syngeneic MLC), and incubated in a concentration of $5 \cdot 10^6$ cells/ml in a volume of 2 mM L-glutamine, $2 \cdot 10^{-5}$ M 2-mercaptoethanol, 10% embryonic calf serum, and antibiotics (100 units/ml each of streptomycin and penicillin), was used as incubation medium. Culture fluids (CF) of 1-2-day MLC were collected at intervals of 1 h, centrifuged at 1000g for 30 min, and then tested for ability to affect macrophage mobility of unimmunized mice in a micromodification of the capillary macrophage migration inhibition test [2]. Macrophage mobility was assessed in absolute values of weight of projection of the migration zone on paper (in mg). CF of syngeneic MLC, and also of monocultures of intact B6 cells, cultured in a concentration of

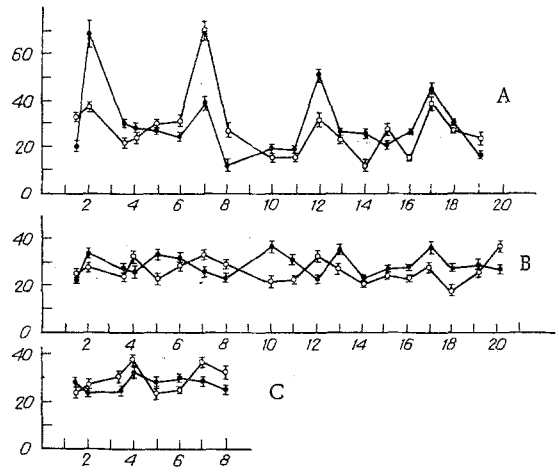

Fig. 1. Macrophage migration in CF of allogeneic (A) and syngeneic (B) MLC and monocultures of B6 spleen cells (C) between 1st and 19th hours of incubation. Abscissa, time from beginning of incubation (in h); ordinate, mean weight of projection of migration zone (in mg standard error of four measurements).

KEY WORDS: mixed lymphocyte culture; inhibition and stimulation of macrophage migration; biorhythms.

Laboratory of Immunochemistry and Diagnosis of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 466-468, April, 1981. Original article submitted May 28, 1980.

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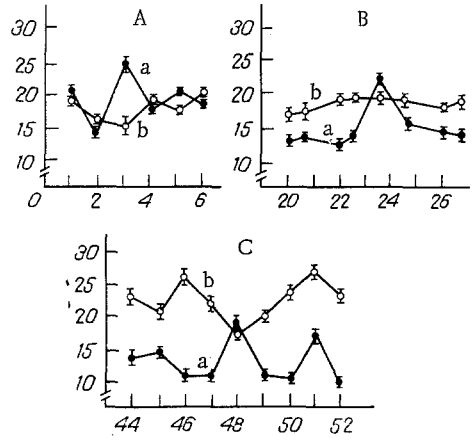

Fig. 2

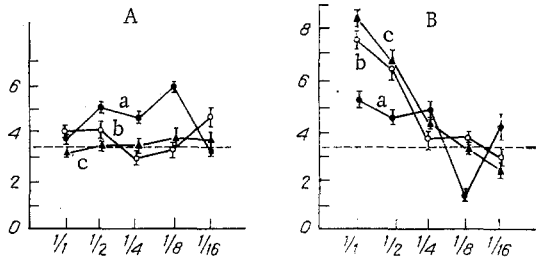

Fig. 3

Fig. 2. Macrophage migration in CF of allogeneic (a) and syngeneic (b) MLC after incubation for 1-6 h (A), 20-26 h (B), and 44-52 h (C). Legend as to Fig. 1.

Fig. 3. Dependence of macrophage migration on dilution of CF of allogeneic (a) and syngeneic (b) MLC and monocultures of spleen cells (c) obtained 24 h (A) and 48 h (B) of incubation. Abscissa, titer of CF; ordinate, mean weight of projection of migration zone (in mg). Broken line indicates level of migration in culture medium.

$2.5 \cdot 10^6$ cells/ml, were tested in the control. Changes in the migration in the allogeneic samples relative to syngeneic were assessed by the macrophage migration inhibition index (MII):

$$\text{MII} = \left(1 - \frac{\text{mean migration zone in allogeneic samples}}{\text{mean migration zone in syngeneic samples}}\right) \times 100\%.$$

EXPERIMENTAL RESULTS

During the first few hours after the beginning of culture a factor or factors sharply stimulating macrophage migration compared with migration of these cells in CF of syngeneic MLC and in monocultures of B6 spleen cells appeared in the CF of the allogeneic MLC. The most characteristic data for two of the six mice studied are illustrated in Fig. 1. Stimulation of macrophage migration in CF of allogeneic MLC was characterized by a sharp peak, appearing regularly with a period of about 5 h, and decreasing in amplitude during the first 24 h of incubation of MLC (Fig. 1A). In CF of syngeneic MLC (Fig. 1B) and in monocultures of B6 spleen cells (Fig. 1C) the peaks of stimulation of migration were smaller in amplitude and did not display the same strict regularity of appearance as in CF of allogeneic MLC. Sharp peaks of stimulation of migration in CF of the allogeneic MLC continued for the first 2 days; toward the end of the 2nd day they began to appear more frequently, with a period of 3-3.5 h (Fig. 2C; the most demonstrative data for one of the six mice tested for each stipulated incubation time of MLC are illustrated in Fig. 2). Conversely, between 42 and 48 h peaks of stimulation in syngeneic MLC appeared less frequently than during the first few hours of MLC and their amplitude increased. Comparison of kinetic curves of absolute values of migration obtained in CF of allogeneic and syngeneic MLC during the first 2 days of incubation revealed a gradual shift of the curve for the allogeneic MLC into the region of inhibition of migration compared with the curve for the syngeneic MLC (Fig. 2). Mean values of MII, calculated during the first 7 h of incubation of MLC, were -9.5 ± 6.3 (28 determinations of MII, four experiments), during 20-26 h they were $+20.1 \pm 5.2$ (22 MII, three experiments) and during 45-52 h, $+33.1 \pm 3.6$ (32 MII, four experiments).

Investigation of the dependence of the absolute area of migration on twofold dilutions of CF obtained after incubation of allogeneic and syngeneic MLC for 18-20 and 44-48 h, and also of monocultures of B6 spleen cells revealed the following relationships (Fig. 3): 1) The dependence was nonlinear in character; 2) the curves of dependence for CF of syngeneic MLC and monocultures were similar to one another and differed from those for CF of allogeneic MLC; 3) during dilution of CF of 24-h allogeneic MLC a peak of stimulation of migration was found, whereas dilution of CF of 48-h cultures gave a peak of inhibition of migration, corresponding to dilutions of 1/8-1/16; 4) migration in CF exceeded in area migration in the culture medium.

The results thus showed that recognition of alloantigens during the first few hours of incubation of MLC is accompanied by the appearance of a factor or factors sharply stimulating macrophage migration. Since the peak of stimulation of migration was found as early as after 2 h of incubation of MLC and since it was characterized by a sudden jump in the absolute values of the area of migration in the course of 30 min, this factor or factors must evidently be present in the cells and be liberated from them on contact with alloantigen. The regular appearance of peaks of stimulation of migration at an interval of not more than 3-5 h indicates the existence of a timing mechanism, controlling the appearance of impulses of stimulation of macrophage mobility, evidently unconnected with cell division. It was shown previously that during stimulation of spleen cells by concanavalin A, MSF appeared on the 3rd-4th day of culture of suppressor cells *in vitro* [8]. These same cells may perhaps produce MSF at earlier stages of incubation of MLC. However, the possibility cannot be ruled out that the stimulation of macrophage migration revealed in the present experiments may be due to activity of cells other than T lymphocytes.

The nonlinearity of dependence of the area of migration on dilutions of CF, the existence of optimal dilutions for stimulation and inhibition of migration, and also the rapid interchange of activity of the factors in the course of incubation of MLC are evidence in support of the presence of a balance of factors in CF with alternative activities: MIF and MSF. Any one of these factors may prove to be identical with the allogeneic factor affecting antibody formation *in vitro* [9].

The early appearance of MIF activity after interaction for 12-16 h between nonimmune lymphocytes and alloantigen in MLC points to the possibility of activation of precursors of MIF producers before development of the primary immune response to other T cell subpopulations. MIF production has recently been demonstrated during culture of nonimmune lymphoid cells with cells of virus-induced tumors [11]. The appearance of lymphokines thus evidently can serve as an indicator of the earliest stages of T cell activation and can reflect the state of natural immunity to transplantation and tumor antigens.

The principles governing the appearance of factors affecting macrophage mobility, found by the writers in the early period of incubation of MLC indicate the existence of a finely balanced and highly sensitive mechanism the biological importance of which may lie in the control of macrophage migration *in vivo* and also in the spatial and temporal organization of these cells during their cooperation with lymphocytes in the course of the immune response.

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