NATURE OF THE CELLS REGULATING MIGRATION INHIBITION FACTOR PRODUCTION IN C57BL/6 MICE WITH LOW RESPONSE TO *Candida albicans* ANTIGEN

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Production of one of the most important lymphokines responsible for interaction between lymphocytes and macrophages, namely migration inhibition factor (MIF), is under genetic control [2, 3]. Lines of mice giving opposite responses to tuberculin [3] and candidin [1] have been discovered; it has been shown, moreover, that genetic differences in the development of the immune response in mice of different genotypes to tuberculin are realized, not at the migrating cell level, but at the level of producers of the factor [3]. At the same time, the regulation of these differences at a given level is a complex, multistage process and is controlled to different degrees by suppressor cells [1, 6].

It was shown previously that C57BL/6 mice react weakly to *Candida albicans*. Accordingly, the aim of the present investigation was to study cellular mechanisms regulating MIF production to *C. albicans* antigen in mice of the low-responding C57BL/6 line.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA and C57BL/6 mice weighing 18-20 g, obtained from the "Rappolovo" and "Stolbovaya" Nurseries, Academy of Medical Sciences of the USSR. To induce an immune response to *C. albicans* antigen mice were immunized intraperitoneally with the glycoprotein antigen of *C. albicans* in a dose of 200 μg per mouse in Freund's incomplete adjuvant in a volume of 0.5 ml. The same glycoprotein antigen in a dose of 10 μg/ml was used to produce MIF. This dose is nontoxic for cells and, at the same time, it is the minimal dose capable of inducing MIF production.

To obtain a supernatant containing MIF, $2 \cdot 10^7$ peritoneal exudate cells, isolated at the peak of the immune response (5th day after immunization), were incubated for 36 h with *C. albicans* antigen (dose 50 μg) in medium RPMI-1640 (from "Flow Laboratories," England) at 37°C. Activity of MIF was determined in the capillary test [3].

The percentage of inhibition of migration (PIM), calculated by the equation:

$$\text{PIM} = 100 - \frac{\text{migration zone with antigen}}{\text{migration zone without antigen}} \times 100\%,$$

was used as the quantitative index of MIF production.

To eliminate suppressor cells the mice were given an intraperitoneal injection of cyclophosphamide in a dose of 200 μg/kg body weight [6]. Spleen cells of immune mice in a dose of $2 \cdot 10^7$ were transferred intravenously to syngeneic recipients, treated with cyclophosphamide as described above two days before the transfer. Spleen cells were transplanted on the 5th day after immunization of the syngeneic donors. After transfer of the cells the recipients were immunized by the scheme described above, and MIF production to specific antigen was determined on the 5th day after immunization.

To eliminate macrophages, the transplantable spleen cells intended for transfer were incubated for 4 h at 37°C in a dose of $2 \cdot 10^7$ in medium 199 with 10% embryonic calf serum and

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TABLE 1. Activity of Supernatants Containing MIF in CBA and C57BL/ 6 Mice

MIF producers (lines of mice)	Target cells (lines of mice)	PIM	P
C57BL/6	C57BL/6	9,6±2,6	>0,05
	CBA	20,5±4,0	
CBA	C57BL/6	39,5±2,6	>0,05
	CBA	38,6±2,7	

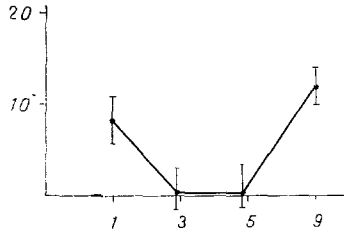

Fig. 1

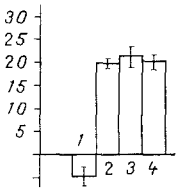

Fig. 2

Fig. 1. Dynamics of formation of suppressors of MIF production in mice of line C57BL/6, with low response to *C. albicans* antigen. Abscissa, time after immunization of donors (days); ordinate, PIM.

Fig. 2. Effect of transplantable cells on MIF production in recipients treated with cyclophosphamide. Abscissa: 1) treatment of transplantable cells with carrageenin, 2) treatment with anti-Thy 1-serum, 3) transfer of spleen cells of intact donors, 4) transfer of bone marrow cells; ordinate, PIM.

with carrageenin in a dose of 400 µg/ml (Viscarin TP-4, from "Cofuku Chemical") [5]. After incubation the cells were washed three times and transplanted into syngeneic recipients in a dose of $2 \cdot 10^7$ cells.

T cells were removed from the suspension of splenocytes by incubation of $5 \cdot 10^7$ cells with 1 ml of mouse anti-Thy 1-serum, diluted tenfold, for 20 min at 37°C (the anti-Thy 1-serum was supplied by the Laboratory of Cellular Immunology, All-Union Research Institute of Tuberculosis, Director M. M. Averbakh). The reagent was then treated with 1 ml of normal (low toxicity) rabbit serum, also diluted tenfold. After incubation for 30 min the cells were washed three times and transplanted [4].

EXPERIMENTAL RESULTS

Lines of mice giving opposite responses to immunization with *C. albicans* antigen were discovered previously: high-responding CBA mice (PIM = 59.4 ± 2.0) and low-responding C57BL/6 mice (PIM = 13.8 ± 2.4). The immune response of mice of both lines reached a peak on the 5th day [1].

The action of supernatants containing MIF in CBA and C57BL/6 mice on adherent peritoneal exudate cells of mice of the same lines was studied (Table 1). It was found that migration of macrophages in mice of oppositely responding lines, after immunization, was inhibited equally by MIF secreted by lymphocytes of CBA mice ($P > 0.05$). Macrophages of mice of these lines were equally sensitive to the action of MIF contained in the medium after incubation of lymphocytes from C57BL/6 mice. However, MIF from CBA mice inhibited migration of macrophages of both lines by a greater degree than MIF from C57BL/6 mice ($P \leq 0.05$). These results suggest that macrophages of lines responding in opposite directions are equally sensitive to MIF. The interlinear differences in MIF production are thus based on different ability of lymphocytes of CBA and C57BL/6 mice to produce the lymphokine.

Injection of cyclophosphamide followed by immunization of the low-responding C57BL/6 line caused a significant increase in MIF production by spleen cells [1]. This confirms the hypothesis regarding the existence of suppressor cells which are eliminated by cyclophosphamide.

The dynamics of generation of suppressor cells in mice of the low-responding line was studied. For this purpose splenocytes of immune mice were transplanted on the 1st, 3rd, 5th,

and 9th days after immunization into donors treated with cyclophosphamide. A significant decrease in production of the factor was obtained on the 3rd and 5th days (PIM was 0.8 ± 2.6 and 1.7 ± 3.5, respectively), but by the 9th day the suppressor effect had disappeared (PIM = 12.9 ± 1.7; Fig. 1).

Transfer of bone marrow cells had no appreciable effect [1] (Fig. 2). It can accordingly be postulated that MIF production by spleen cells to specific antigen is controlled mainly by cells of thymic origin.

Preliminary incubation of the transplantable spleen cells with carrageenin did not abolish their suppressor effect (PIM = −4.3 ± 3.7; Fig. 2). Hence it can be concluded that macrophages are not suppressors in this system. Meanwhile treatment of the transplantable cells with anti-Thy 1-serum led to abolition of suppression (PIM = 19.9 ± 1.1; Fig. 2), thereby confirming the role of T cells in the regulation of MIF production by spleen cells.

To elucidate the role of antigen in suppressor formation under these experimental conditions, syngeneic spleen cells from intact mice were transferred into mice treated with cyclophosphamide (PIM = 23.7 ± 3.8). The results of this investigation confirmed that suppressors are formed under the influence of specific antigen, for intact cells were unable to induce suppression of MIF production (Fig. 2).

These results suggest that low ability to produce the mediator in response to *C. albicans* antigen is due to the existence of specific suppressors, sensitive to cyclophosphamide, and that interlinear differences can be explained by different activity of suppressor cells of thymic origin in mice of lines responding in opposite directions. This is an interesting result in connection with the further study of the regulatory mechanisms of lymphokine production and also for phenotypic correction of the immune response.

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