INTERLINEAR DIFFERENCES IN MICE IN PRODUCTION OF MIGRATION INHIBITION FACTOR TO *Candida albicans* ANTIGENS

K. V. Vorob'ev, L. V. Gankovskaya,
L. V. Koval'chuk, and L. F. Shabanova

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The study of the genetic factors affecting the immune response to various antigens has attracted the attention of many scientists. Recently the genetic control of a macrophage migration inhibition factor (MIF) to tuberculin in mice has been studied [2, 3]. This reaction is the analog of delayed-type hypersensitivity (DTH) *in vitro*. It has been shown that lines of mice with different haplotypes respond to different degrees to this antigen. For instance, line C57BL/6j is high-responding, but line CBA is low-responding. Interlinear differences also have been described in the development of DTH to tuberculin in other laboratory animals [6, 8]. These data suggest that the cellular immune response to tuberculin is controlled genetically and that high MIF production in mice is a faculty with dominant type of inheritance; several genes, one linked with the principal histocompatibility complex, others not so linked, have been coded [3]. It is therefore interesting to study MIF production in mice to antigens of other pathogenic microorganisms and, in particular, to the widely distributed fungus *Candida albicans*. Candidiasis of the skin and mucous membranes, caused by this fungus, is known to have an autosomal-dominant type of inheritance [10]. Types of inheritance have also been established for other diseases of this group [4, 7, 11].

In the investigation described below the time course of MIF production was studied in mice sensitized to glycoprotein and polysaccharide antigens isolated from *Candida albicans*.

EXPERIMENTAL METHOD

Inbred mice of lines CBA ($H\text{-}2^k$), C57BL/6j ($H\text{-}2^b$) and (CBA × C57BL/6j)F_1 hybrids, obtained from the "Stolbovaya" Pure-Line Animal Nursery, Academy of Medical Sciences of the USSR, were used. To induce DTH two types of antigens isolated from the pathogenic fungus *Candida albicans* in the Department of Deep Mycoses, State Postgraduate Medical Institute, Leningrad were selected – polysaccharide and glycoprotein antigens. The polysaccharide antigen, extracted from cells of the fungus by an aqueous alcoholic solution of β-naphthol, followed by precipitation with ethyl alcohol, consists mainly of mannose and glucose with a small quantity of protein. This antigen is nontoxic. The glycoprotein antigen, also used in the work, was obtained from *C. albicans* cells by extraction with water during autoclaving, followed by purification on cation-exchange gel Sephadex G-25. It differs from the polysaccharide antigen in having a higher protein content.

Mice were immunized intraperitoneally with both polysaccharide and glycoprotein antigens together with Freund's incomplete adjuvant (200 μg of antigen in 0.4 ml per mouse). Spleen cells were obtained by the usual method from the 1st through the 17th day and MIF production was determined by the direct macrophage migration inhibition capillary test of George and Vaughan [5] with some modifications [2]. The same substances were used as antigen as for immunization, in an optimal dose of 10 μg/ml, nontoxic for cells. The percentage of inhibition of migration (PIM), determined by the equation:

$$\text{PIM} = \frac{\text{Zone of migration of cells with antigen}}{\text{Zone of migration of cells without antigen}} \cdot 100\%,$$

Department of Immunology, N. I. Pirogov Second Moscow Medical Institute. Department of General and Applied Immunology, S. M. Kirov State Postgraduate Medical Institute, Leningrad.
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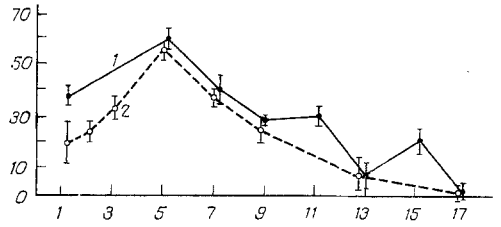

Fig. 1

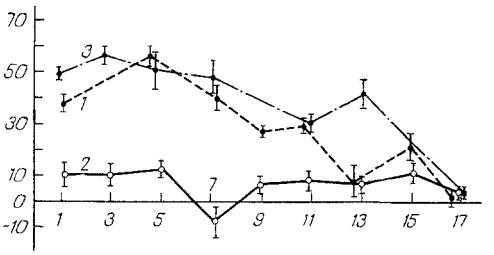

Fig. 2

Fig. 1. Time course of MIF production by CBA mouse lymphocytes. Here and in Fig. 2: abscissa, time after immunization (in days); ordinate, percent inhibition of migration. 1) MIF production to glycoprotein antigen, 2) MIF production to polysaccharide antigen.

Fig. 2. Time course of MIF production to glycoprotein antigen in mice of different lines. 1) Time course of MIF production in CBA mice, 2) the same in C57BL/6j mice, 3) the same in (CBA × C57BL/6j)F_1 hybrids.

was used as the quantitative index of MIF production. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The scheme of immunization and dose for testing the cellular immune response to *Candida albicans* antigens were worked out. A dose of 200 μg antigen per mouse was chosen for immunization, as the smallest dose evoking an immune response, detectable in our experiments from the first through the seventeenth days. A dose of 10 μg antigen/ml was chosen for testing the cellular immune response. Splenic lymphocytes were producers of MIF, and macrophages were the migrating cells.

The time course of MIF production after intraperitoneal injection of polysaccharide and glycoprotein antigens of CBA mice is illustrated in Fig. 1. By the 2nd day splenic lymphocytes were producing lymphokines in both cases. The maximal production of MIF was observed on the 5th day (PIM was 59.4 ± 4.0 and 57.8 ± 6.8 respectively). MIF production gradually declined until the 13th day, and on the 17th day after immunization it was completely absent. Incidentally, the curves of MIF production to glycoprotein antigen did not differ significantly from those of MIF production to polysaccharide antigen.

The specificity of MIF production to the antigen used was demonstrated in experiments in in which another antigen was added for testing production of the factor, in particular, tuberculin. Under these circumstances production of the mediator was not found (PIM = 97.6 ± 9.2).

The next step of the work was to determine production of mediator by mouse lymphocytes to glycoprotein antigen in the direct capillary test in C57BL/6j mice (Fig. 2). MIF production in this line was almost indistinguishable from that in intact mice throughout the 17 days after immunization and maximal production of the factor was found on the 5th day (PIM = 13.8 ± 2.4); it then declined gradually and ceased by the 17th day.

The investigation thus showed that CBA ($H\text{-}2^k$) mice are highly responsive to *C. albicans* antigens, whereas C57BL/6j ($H\text{-}2^b$) mice are low-responding and maximal production of the factor in these lines was observed at the same time, namely on the 5th day after immunization (PIM was 54.4 ± 4.0 and 13.8 ± 2.4 respectively).

The presence of interlinear differences in MIF production is evidence that these differences are genetically based. To discover the character of inheritance of this trait, the time time course of MIF production by (CBA × C57BL/6j)F_1 mouse lymphocytes was studied. The character of the curve of its time course practically coincided with that of highly responsive CBA mice, the only difference being that production of the factor reached a maximum on the 3rd day after immunization (PIM = 51.1 ± 2.8; Fig. 2). First generation hybrids [(CBA × C57BL/6j)F_1] thus respond to glycoprotein antigen in the same way as the highly responsive CBA line.

The results demonstrated that MIF production is a genetically controlled process. Lines of mice (CBA and C57BL/6j) responding in opposite directions to *C. albicans* antigen have been found. It has also been shown that hybrids responded to the antigen used in the same way as the highly responsive CBA ($H\text{-}2^{K}$) line. Probably a high level of MIF production is inherited as a dominant trait, but without further analysis it is impossible to determine the number of genes which control this process.

The fact of genetic control of MIF production to *C. albicans* antigens may be important in the development of approaches to the evaluation of human sensitivity to the agent of candidiasis. Knowledge of the mechanisms of genetic control of MIF production to the antigens studied is important in order to reveal the character of action of these substances in the various allergic tests.

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