DEVELOPMENT OF THE CELLULAR IMMUNE RESPONSE TO TUBERCULIN IN MICE OF DIFFERENT GENOTYPES

L. V. Koval'chuk, E. V. Sokolova, and L. V. Burtseva

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Inbred CBA and C57BL mice and (CBA × C57BL)F_1 hybrids were immunized intraperitoneally with Freund's complete adjuvant and the production of macrophagal migration inhibiting factor (MIF) by lymphocytes in different situations was investigated. Lymphocytes were activated to produce MIF in a certain order: cells of the peritoneal exudate first, then lymph node cells and, finally, spleen cells. Maximal MIF production by lymphocytes of the peritoneal exudate was obtained in C57BL mice, and it occurred earlier (on the 3rd day after immunization). Increased spontaneous migration of macrophages also was observed after immunization and it was more marked in the CBA mice.

KEY WORDS: *Tuberculin; cellular immunity; macrophagal migration inhibiting factor; genetic control.*

It has now been clearly established that antibody formation is a genetically determined process [1-3]. However, the principles governing genetic control over the development of reactions of the delayed hypersensitivity type have received little study. There have been only a few investigations which have demonstrated interlinear differences in the reactions of cellular immunity in mice [11, 12], guinea pigs [8], rabbits [10], and chickens [7].

The object of this investigation was to study the dynamics of production of one of the mediators of cellular immunity, namely macrophagal migration inhibiting factor (MIF), in response to tuberculin immunization of CBA, C57BL, and (CBA × C57BL)F_1 hybrids. Inbred CBA and C57BL mice differ with respect to the strong histocompatibility locus, ability to produce antibodies against various corpuscular antigens, such as sheep's red cells and leptospires [1-3], and also with respect to the number of cells forming rosettes with sheep's red cells [9].

EXPERIMENTAL METHOD

Inbred CBA ($H\text{-}2^k$) and C57BL ($H\text{-}2^b$) mice and (CBA × C57BL)F_1 hybrids weighing 15-20 g were immunized by a single intraperitoneal injection of Freund's complete adjuvant in a dose of 500 µg. The total volume of mixture injected was 0.4 ml per mouse. At various times from the 1st to the 17th day MIF production by sensitized lymphocytes was studied by the direct capillary macrophagal migration inhibition test [6]. The producers of MIF were lymphocytes contained in peritoneal exudate, inguinal and axillary lymph nodes, and the spleen of the immunized mice. The migrating cells were peritoneal exudate macrophages of intact or immunized mice. To obtain peritoneal exudate cells the mice were given an intraperitoneal injection of 1.5 ml of 2% peptone solution. Suspensions of cells from peritoneal exudate, spleen, and lymph nodes were prepared by the usual method [4, 5]. Glass capillary tubes were filled: 1) with peritoneal exudate cells of immunized mice; 2) with spleen cells of immunized mice; and 3) with a mixture of cells from lymph nodes of immunized mice and peritoneal exudate cells of intact mice in the ratio of 1:5. The capillary tubes were centrifuged at 200g for 10 min and then placed in chambers each containing 100 units penicillin, 100 units

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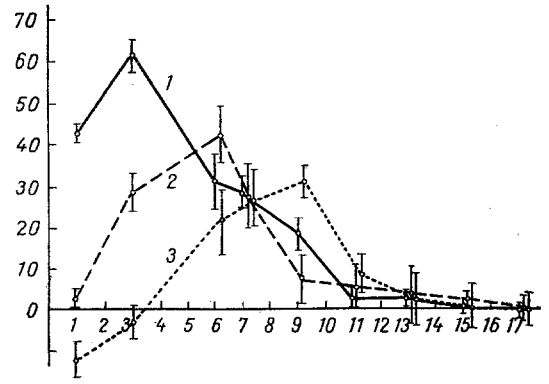

Fig. 1

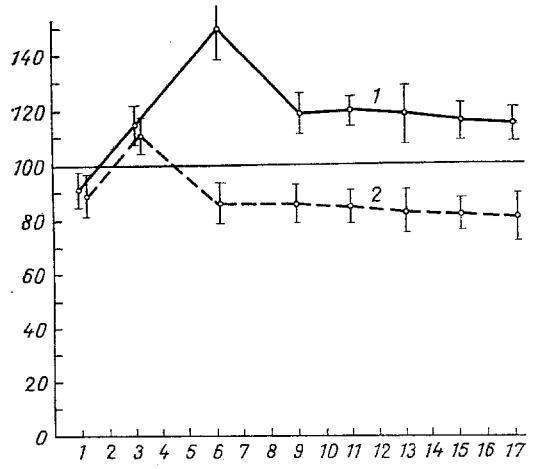

Fig. 2

Fig. 1. Dynamics of MIF production by lymphocytes of peritoneal exudate, lymph nodes, and spleen of C57BL mice immunized with tuberculin: 1) peritoneal exudate cells of immunized mice; 2) mixture of lymph node cells of immunized mice with peritoneal exudate cells of intact mice; 3) spleen cells of immunized mice. Ordinate, here and in Fig. 3, percentage of inhibition of migration; abscissa, here and in Figs. 2 and 3, time after immunization (in days).

Fig. 2. Spontaneous migration of peritoneal exudate cells of C57BL (1) and CBA (2) mice immunized with tuberculin. Ordinate, percentage migration.

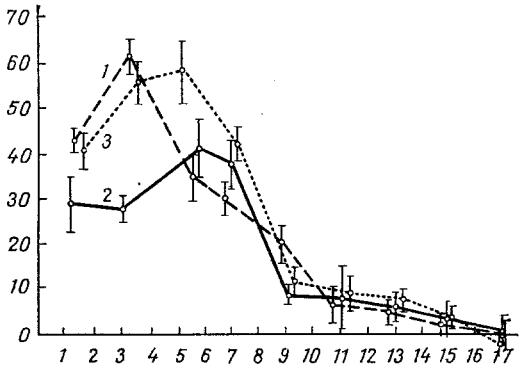

Fig. 3. Degree of inhibition of migration of peritoneal exudate macrophages of mice of various strains: 1) C57BL, 2) CBA, 3) (CBA × C57BL)F_1 mice.

streptomycin, and 100 μg tuberculin to 1 ml medium 199. This was the optimal dose of tuberculin and was nontoxic for the cells. Medium not containing antigen was added to the control chambers. The zones of migration were projected by the "Mikrofot" 5PO-1 apparatus on paper. The zone of migration was cut out and the percentage inhibition of migration (PIM) calculated by the equation

$$\text{PIM}=100\%\ \frac{\text{Zone with antigen}}{\text{Zone without antigen}}\times 100\%.$$

A control group of animals also was used to assess spontaneous migration of the peritoneal exudate cells at various times after immunization. The mean number of capillary tubes was eight (in the experimental and control series respectively). The results were subjected to statistical analysis with the use of Student's criterion.

EXPERIMENTAL RESULTS

Comparison of MIF production by sensitized lymphocytes in different parts revealed a definite pattern of their activation as effectors of the cellular immune response (Fig. 1).

For instance, MIF production by lymphocytes of the peritoneal exudate of C57BL mice began earlier (3rd day) and was more intensive (PIM 62.5±3.38%). Lymph node lymphocytes were activated somewhat later, on the 6th day (PIM 42.4±6.9%) and, finally, splenic lymphocytes were activated on the 9th day (PIM 30.8±3.8%). A similar dynamics of development of the immune response also was found in the other strains of mice.

In the next series of experiments the dynamics of spontaneous migration of peritoneal exudate cells of immunized mice was studied, i.e., the ability of the cells to leave the capillary tube under optimal conditions of cultivation without the addition of specific antigen to the medium was determined.

During development of the immune response changes in the spontaneous migration of peritoneal exudate cells were observed (Fig. 2). In CBA mice, for instance, on the 6th day after immunization spontaneous migration was observed to be stimulated by 51±10.2% compared with the control (migration of intact macrophages was taken as 100%). In immunized C57BL mice spontaneous migration was increased only very slightly, by 12±6.3%, on the 3rd day after immunization.

Significant differences in the value of PIM were found by investigation of the migrating ability of peritoneal exudate cells of CBA and C57BL strains of mice and (CBA × C57BL)F_1 hybrids (Fig. 3). The highest values of PIM were found in C57BL mice (62.2±3.38%) on the 3rd day after immunization, whereas in CBA mice (40.8±6.7%) they did not occur until the 6th day. PIM for the F_1 hybrid mice was 58.2±6.5% on the 5th day.

Peritoneal exudate cells of CBA and C57BL mice thus differed in the degree of inhibition of their migrating ability in response to tuberculin. Inbred C57BL mice were more highly reactive than CBA mice. These differences may be due to differences in the ability of CBA and C57BL mice to accumulate MIF-producing cells in the peritoneal exudate or differences in the ability of their lymphocytes to synthesize MIF. However, in this particular test the possibility that macrophages of the different strains of mice differ in their sensitivity to MIF likewise cannot be ruled out.

With the aid of the capillary method spontaneous migration of peritoneal exudate cells could also be estimated at different times after immunization. Spontaneous migration of peritoneal exudate cells was more active in immunized CBA mice, possibly as a result of both an increase in the number of macrophages in the peritoneal exudate after immunization in this strain of mice and an increase in their functional activity.

When the results shown in Figs. 2 and 3 are compared it must be remembered that the maximal degree of inhibition of migration of peritoneal exudate macrophages was observed at the time of their most active spontaneous migration. For instance, maximal MIF production in CBA mice (the 6th day after immunization) coincided with the most marked spontaneous migration. The possibility cannot be ruled out that these activated macrophages are more sensitive to the reception of MIF, and that this is the reason for the highest degree of inhibition of migration.

In response to intraperitoneal injection of the antigen, cellular immunity mediated by lymphocytes from different parts of the body differed in its strength and the time of its development. For instance, peritoneal exudate lymphocytes were most active as regards MIF production, followed by lymphocytes of the lymph nodes and spleen; the reason for this pattern was evidently increased migration of lymphocytes into the peritoneal cavity.

The fact that different strains differ in the development of the cellular immune response to tuberculin could play a role in the development of methods of evaluation of individual sensitivity to *Mycobacterium tuberculosis* in man.

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GRAFT VERSUS HOST REACTION IN RECIPROCAL COMBINATIONS OF MOUSE STRAINS DIFFERING IN THEIR H-2 HISTOCOMPATIBILITY COMPLEX

D. N. Mayanskii

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Spleen cells from C57BL mice injected intraperitoneally into newborn CBA recipient mice in doses of $0.5 \cdot 10^7$, $1 \cdot 10^7$, $2 \cdot 10^7$ induced runt disease in an acute form, from which 43, 86, and 95% of the recipients respectively died in the course of two or three weeks. Preliminary immunization of the C57BL donors with CBA isoantigens led to a marked increase, whereas immunization with "foreign" antigens (sheep's red cells) led to weakening of the reactions. With the reciprocal combination of strains runt disease followed a course 4-5 times less active and there was no "preimmunization effect." In the combination C57BL→CBA the reaction was accompanied by proliferation of pyroninophilic monocytes and by destruction of the splenic follicles, whereas in the combination CBA→C57BL their formation was delayed and no appreciable accumulation of blast cells took place in the zone of the follicle.

KEY WORDS: *Graft versus host reaction; runt disease; transplantation*

The graft versus host reaction (GVHR) resulting from transplantation of nonsyngeneic immunocompetent cells or their precursors into an immunologically inert recipient in the embryonic or early postnatal period of development, has been called "runt disease" [4]. It has been widely used in recent years as a model with which to estimate the functional activity of various categories of lymphocytes [3] and their interaction in reactions of transplantation immunity [5].

The course of runt disease as one form of the GVHR was studied in the investigation described below in relation not only to the degree of genetic differences between donor and recipient, but also to their reciprocal position in the combination of strains.

EXPERIMENTAL METHOD

Inbred mice of strains CBA ($H\text{-}2^k$) and C57BL/6 ($H\text{-}2^b$) were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. To induce the GVHR, donors' spleen cells, washed three times and suspended in 0.05 ml of medium No. 199, were injected intraperitoneally into the recipients during the first 24 h after birth. In series I, spleen cells of the C57BL genotype were injected into CBA recipients. In the experiments of series II the reciprocal combination of strains was used. For alloimmunization the donors were given an

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