The intermediate value of the immune response found in the F_1 hybrids compared with responses of the parental lines is evidence against the hypothesis of simple dominance of the characteristic of strength of the immune response to the antigen used. Inheritance of the ability to give an immune response to MPAA is evidently codominant in character, as has also been shown for several other antigens [6].

When discussing the possible causes of the low immune response of C57BL/6 mice to MPAA it must be noted that these data are evidence against a role of antigenic mimicry in determination of the level of the immune response in this test system. Considering data in the literature [4] obtained by a study of control of the immune response to pneumococcal polysaccharide, it can be postulated that the genetic defect in C57BL/6 mice is expressed as reduced activity of T amplifiers or increased generation of specific T suppressors. Another possible cause of the low level of the immune response in C57BL/6 mice may be the small number of clones of B cells carrying specific Ig receptors, and also the insufficient ability of B cells to differentiate into antibody-producing cells under the influence of antigen and (or) regulatory factors. In further experiments the writers will aim to determine the cellular level of expression of genes of the immune response in mice responding in an opposite manner to MPAA.

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SPONTANEOUS AND CONCANAVALIN A-INDUCED PRODUCTION OF LEUKOCYTE MIGRATION INHIBITION FACTOR BY HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Much attention is now being paid to the control of production of lymphokines and, in particular, of factors influencing migration. Such factors can be produced under the influence of various stimuli, and also spontaneously, under normal conditions [1, 2] and in various diseases [6-8, 11]. It has been shown that cultures of T cells contain an inhibitor of the production of microphage migration inhibition factor (MMIF) [9]. It can be tentatively suggested that a regulatory role in MMIF production is played by suppressor cells [5]. However, the factors and conditions responsible for regulation of production of leukocyte migration inhibition factor (LMIF) have received little study.

The object of this investigation was to study LMIF production by human peripheral blood lymphocytes (PBL) *in vitro*, spontaneously and under the influence of the mitogen concanavalin A (con A).

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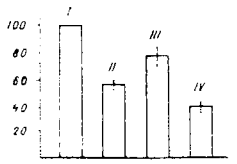

Fig. 1. Spontaneous and con A-induced production of LMIF by PBL. Abscissa: I) spontaneous migration, II) spontaneous LMIF production, III) incubation with con A in a dose of 2.5 μg/ml for 1 h, IV) incubation with con A in a dose of 5-10 μg/ml for 4 h; ordinate, MI (in percent, M ± m, control — spontaneous migration).

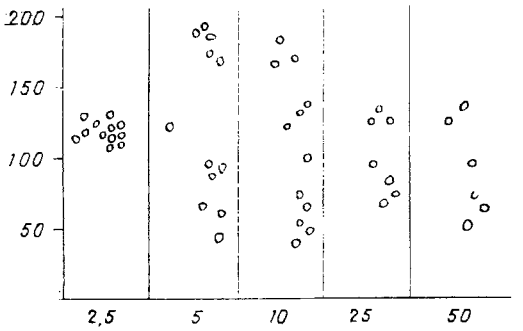

Fig. 2. Effect of dose of con A on LMIF production by PBL. Abscissa, dose of con A (in μg/ml); ordinate, MI (in percent, control — zone of migration in supernatant of culture without mitogen).

EXPERIMENTAL METHOD

PBL were isolated by centrifugation of heparinized healthy human blood in a Ficoll—Verografin density gradient [4]. The cell concentration was adjusted to 2 million/1 ml. To study spontaneous LMIF production the PBL were cultured for 24 h at 37°C in medium 199 with the addition of penicillin and streptomycin. The cells were then sedimented by centrifugation at 200 g for 10 min and 1 ml of supernatant was added to the test system. To select optimal conditions for LMIF production under the influence of the mitogen, PBL were incubated with various doses of con A (2.5-50 μg/ml) for 1 or 4 h at 37°C. The cells were then washed three times with Hanks' solution and mixed with medium 199 for 20-24 h at 37°C. Cultures in which no mitogen was added or with which the same manipulations were carried out as with the experimental cultures served as the controls. The cells were then sedimented by centrifugation and 1 ml of supernatant was added to the test system.

The effect of the supernatant on leukocyte migration was studied in the capillary tube test [10]. Leukocytes were isolated from healthy human peripheral blood. Capillary tubes were filled with the cells thus obtained, and placed in wells containing medium 199 to estimate spontaneous migration or containing the test supernatant of the cultures. Results were read after culture for 20 h at 37°C and the migration index (MI) was determined [10] and expressed in percent:

$$MI. = \frac{P_1}{P_2} \cdot 100\ \%,$$

where P_1 is the migration zone in the experiment and P_2 the migration zone in the control. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The supernatant of PBL cultures not stimulated by the mitogen considerably inhibited ($P < 0.01$) spontaneous leukocyte migration. MI was 58.97 ± 3.04% (Fig. 1, II).

Some workers have reported instability of MMIF and LMIF production during stimulation of lymphocytes by con A [2, 5]. In the present investigation, when the effect of the dose of mitogen and time of incubation with it on LMIF production was studied the following result was found. On stimulation of the PBL culture with con A in doses of 5, 10, 25, and 50 μg/ml for 1 h considerable instability of LMIF production was observed compared with the control without mitogen (Fig. 2). If the dose of mitogen was reduced to 2.5 μg/ml the extent of migration in supernatant of the culture with con A became more constant (Fig. 2). It was much higher ($P < 0.05$) than in culture without mitogen but lower ($P < 0.05$) than the spontaneous migration (Fig. 1, III). Under these conditions LMIF production was thus less than spontaneous production of the factor, i.e., the latter was depressed but not completely suppressed. Fox et al. [5] suggested that suppressor cells play a regulatory role in MMIF production. The possibility cannot be ruled out that if a small dose of mitogen is used, suppressors of production or suppressors of producer cells of LMIF are stimulated.

With an increase in the incubation time with con A in doses of 5 and 10 μg/ml to 4 h, LMIF production was stabilized. MI did not differ significantly ($P < 0.05$) in these two groups, and they were therefore combined into one group. Not only marked inhibition of spontaneous migration ($P < 0.01$), but also a greater degree ($P < 0.05$) of LMIF production was observed compared with spontaneous production of the factor (Fig. 1, IV). Inhibition of spontaneous migration evidently reflects the combined effect of LMIF, produced spontaneously and also in response to induction by con A.

Under certain conditions of incubation con A thus has a stimulating effect on activity of cells producing LMIF, but the decrease in LMIF production associated with a decrease in the dose of the mitogen and of the time of incubation with it evidently reflects activation of suppressor cells. The simultaneous study of factors influencing leukocyte migration, produced spontaneously and in response to induction by different doses of con A can be used as a model with which to study immunologic reactivity under normal and pathological conditions. A more detailed study of cells participating in the control of production of factors influencing migration will lead to a better understanding of the nature and the biological role of these factors.

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