INTERLINEAR DIFFERENCES IN CHANGES IN 5-NUCLEOTIDASE ACTIVITY OF MOUSE MACROPHAGES IN RESPONSE TO IMMUNOSTIMULATION

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KEY WORDS: 5-nucleotidase; peritoneal macrophages; immunostimulants; H-2 complex

A central problem in modern immunology is genetic control of the immune response. The process of antibody formation is genetically determined [5]. Interlinear differences have been found in response to cellular immunity in mice [3, 6].

Immunogenesis is based on biochemical mechanisms which bring about adaptive changes in metabolism. Accordingly, interest in the study of metabolic aspects of the immunologic response has increased considerably in recent years, with particular reference to the study of 5-nucleotidase (5-NT), one of the principal enzymes of purine catabolism.

Lowering of 5-NT activity in macrophages is a biochemical marker of activation of these cells [9]. The immunostimulant action of various substances is based on their activating effect on cells of the mononuclear phagocytic system, which is manifested as a decrease in 5-NT activity [7].

To discover the mechanism of action of immunostimulants, the genetic control of immunostimulation is an important problem, but the principles of genetic control of this metabolic feature have virtually not been studied.

The aim of this investigation was to study the effect of immunostimulants on 5-NT activity in peritoneal exudate macrophages (PEM) from different lines of mice.

EXPERIMENTAL METHOD

Inbred male mice aged 3 months, of the following strains, were used: CBA ($H\text{-}2^k$), AKR ($H\text{-}2^k$) C3H/He ($H\text{-}2^k$), BALB/c ($H\text{-}2^d$), C57Bl/6 ($H\text{-}2^b$), C57BR ($H\text{-}2^b$), A ($H\text{-}2^a$), (CBA × C57Bl/6)F_1 hybrids (F_1), and noninbred mice.

5-NT activity was determined 24 h after subcutaneous injection of the immunostimulants by a modified method [7] in PEM. Macrophages were obtained by the method [1]. The immunostimulants salmosan (a polysaccharide of microbial origin, obtained at the Laboratory of Natural Immunity, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, by M. A. Tumanyan, N. G. Sinilova, and A. P. Duplishcheva), tuftsin, and rigin (synthetic tetrapeptides, obtained at the Institute of Bioorganic Chemistry, Academy of Sciences of the Latvian SSR, by G. I. Chipons), were injected in a single dose of 100 g per mouse. Animals receiving isotonic sodium chloride solution served as the control. The mice were killed by cervical dislocation. Samples were taken at the same times during the morning. The experiments were done in the fall and winter. The experimental results were subjected to statistical analysis by Montsevichyute-Éringene's method [4].

EXPERIMENTAL RESULTS

The results of measurement of 5-NT activity in PEM 24 h after subcutaneous injection of the immunostimulants into mice of the various lines are shown in Fig. 1. Under the influence of salmosan, the most effective of the immunostimulants used, no decrease in 5-NT activity was found in PEM from CBA and C3H/He mice. Tuftsin and rigin likewise caused no decreased in enzyme activity in PEM from CBA mice. Injection of the immunostimulants in AKR, BALB/c, C57Bl/6, and C57BR mice, including the F_1 hybrid and noninbred mice, reduced 5-NT activity

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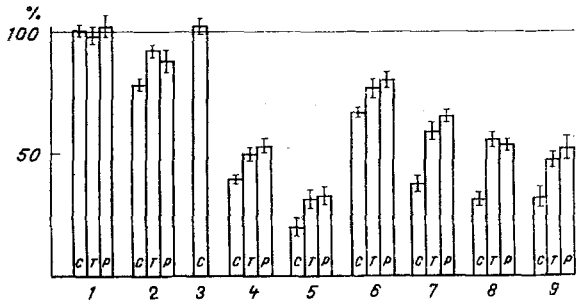

Fig. 1. 5-NT activity in PEM from mice of various lines 24 h after subcutaneous injection of immunostimulants (in % of control, M ± m·t). 1)CBA; 2) AKR; 3) C3H; 4) BALB/c; 5) C57Bl/6; 6) C57BR; 7) A; 8) F_1; 9) noninbred mice; s) salmosan; t) tuftsin, r) rigin. 1, 2, 4-9) Thirty mice used with each substance; 3) 20 mice used.

in PEM, and the decrease was greatest after injection of salmosan.

The decrease in 5-NT activity observed in PEM indicates activation of these cells in response to immunostimulation. The degree of decrease of 5-NT activity in PEM differs, incidentally, in mice of different lines: the greatest decrease in enzyme activity after injection both of salmosan and of tuftsin and rigin was observed in C57Bl/6 mice, the smallest in AKR mice.

The results of this investigation thus show that interlinear differences are found in the change in 5-NT activity in PEM following subcutaneous injection of immunostimulants.

Comparative experiments to study 5-NT activity in inbred mice of different lines did not suggest the presence of linkage of the genes responsible for variation of the enzyme with the H-2 complex of the main histocompatibility system. For instance, among mice with the $H\text{-}2^k$ haplotype there are lines not reacting by a change in 5-NT activity (CBA, C3H), and others responding weakly by a small decrease in enzyme activity (AKR). Among mice with the $H\text{-}2^b$ haplotype, C57Bl/6 mice gave a strong response, C57BR mice an intermediate response.

Comparison of levels of 5-NT activity in intact mice of different lines is interesting. In CBA and C3H mice ($H\text{-}2^k$ locus) 5-NT activity in PEM was least, namely 32 ± 1.67 and 37 ± 2.64 μg phosphorus/10^7 cells/h respectively at 37°C. The highest enzyme activity was found in intact C57Bl/6 ($H\text{-}2^b$) mice: 58 ± 2.03 μg phosphorus/10^7 cells/h at 37°C. It can be tentatively suggested that the level of 5-NT activity in PEM from unstimulated mice reflects the natural functional activity of these cells. The low level of enzyme activity in the CBA and C3H mice points to higher natural activity of the macrophages in these animals than those of mice of the other lines studied. This hypothesis is in good agreement with data on the correlation between 5-NT activity, the level of natural activity of the macrophages, and the level of resistance of the mice to listerellosis [10]. Comparison of the lysosomal enzyme levels and other parameters of macrophage activity in mice of different lines also shows that the macrophages in CBA and C3H mice are in a more active state than those in C57Bl/6 mice [2, 8, 11].

The character of the effect of immunostimulants on 5-NT activity in PEM evidently depends on the initial level of functional activity of the macrophages in intact mice. For instance, the greatest decrease in 5-NT activity in PEM, i.e., maximal activation compared with intact animals, was induced by the immunostimulants in C57BL/6 mice, whose resident macrophages exhibit low functional activity.

The results of this investigation thus showed that the character of action of immunostimulants on 5-NT activity in PEM is determined by the initial natural level of activity of this enzyme in these cells in intact animals, and that this metabolic feature is genetically determined.

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GENETIC ANALYSIS OF HUMAN IMMUNITY PARAMETERS

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Immunologic methods are widely used to study the genetic polymorphism of human populations. They are used in the study of human antigenic systems, such as blood groups, histocompatibility antigens, and immunoglobulins and their correlates with other immunologic, and also morphophysiological and biochemical features [6, 9, 15]. Their use as genetic markers, which has become traditional in population-genetic investigations, must be complemented by the study of the role of hereditary factors in the total phenotypic variation of immunity parameters in man which, in their nature, are multifactorial quantitative features. This problem can be solved by investigations on twins, and also by the genetic analysis of the structure of links between concrete features in pairwise comparisons of the relatives with different degrees of kinship, in the course of a genetic-epidemiologic investigation.

The few investigations already undertaken, namely on twins, have pointed to the important role of genetic factors in the formation of a particular level of certain immunologic parameters [4, 13, 14]. However, there have been virtually no genetic-epidemiologic investigations of the immunologic status in man.

In the investigation described below the authors have attempted for the first time to assess the role of hereditary factors in the total variation of certain immunologic characters in one of the circumpolar populations, a description of which was given previously [7].

EXPERIMENTAL METHOD

A sample representative for both sex and age, consisting of 372 persons (181 men and 191 women), aged 18-50 years, was studied. Eighty parent-child, 41 sib-sib, and 45 parent-parent pairs were formed from them.

The following immunologic parameters were determined by the usual methods: the relative numbers of T- and B-rosette-forming cells (T- and B-RFC) [10], the serum complement level [8], the serum β-lytic and lysozyme activity [1, 5], IgA, IgM, and IgG levels according to Mancini, and the titer of normal (heterophilic) antibodies to sheep's red blood cells.

Interfamilial correlation analysis included calculation [2, 3] of coefficients of phenotypic correlations in parent-child (r_{pc}), sib-sib (r_{ss}), and parent-parent (r_{pp}) pairs, averaged by Fisher's z-transformation. For immunologic parameters with significantly different r_{pp} values, r_{pc} and r_{ss} were corrected for assortative mating [2]. To assess genetic links between characters the coefficient of cross-correlation r^+ was used [3]:

$$r^+ = \frac{\sum_n^n (x_{np} - \bar{x}_p)(y_{nc} - \bar{y}_c) + \sum_n^n (y_{np} - \bar{y}_p)(x_{nc} - \bar{x}_c)}{\sigma x \, \sigma y 2(N-1)},$$

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