PHENOTYPIC CORRECTION OF THE IMMUNE RESPONSE WITH L-TYROSINE IN MICE OPPOSITE IN THEIR RESPONSE TO SHEEP'S RED BLOOD CELLS

V. V. Lebedev, M. I. Titov,
G. F. Maksimova, A. P. Portnova,
A. S. Molokoedov, E. V. Zaitsevskaya,
and G. N. Khlyabich

UDC 612.017.1:547.466

KEY WORDS: thymic polypeptides; amino acids; antibody-forming cells; IgM.

The strength of the immune response to the same antigen varies considerably in animals of different lines and is controlled by the system of genes of the immune response — the Ir-genes [6]. Ability to produce antibody-forming cells (AFC) in response to sheep's red blood cells (SRBC) in mice differs: the minimal immune response is observed in C57BL/6 mice, the maximal in CBA and (CBA × C57BL)F_1 mice [1]. By the use of an experimental model of mice responding in opposite directions to SRBC, it has been shown that several synthetic adjuvants induce stimulation of AFC production only if injected into mice with low-reacting genotypes [1]. The possibility of converting low-reacting into high-reacting individuals by injecting natural biological regulators into the animals was studied. For this purpose two groups of substances were compared: L-tyrosine and thymic polypeptides (Tactivin) — with respect to their ability to effect phenotypic correction of the strength of the immune response in CBA and C57BL/6 mice, reacting in opposite directions to SRBC.

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL/6 and CBA mice weighing 14-16 g. The animals were immunized by a single intramuscular injection of a suspension of SRBC containing 5×10^6 cells in a volume of 0.5 ml of 0.14 M NaCl solution. The number of AFC in the spleen of the mice was counted on the 5th day after immunization by the method in [4]. AFC producing hemolysins of the IgM class were counted. The preparations were injected in a single dose of 1, 10, or 100 μg, 1 h before immunization, in a volume of 0.5 ml of 0.14 M NaCl solution. Before immunization animals of the control group were given the same volume of isotonic NaCl solution. The total number of AFC in the spleen was counted per 10^6 karyocytes. The index of stimulation (IS-AFC) was determined as the ratio of the number of AFC in the experiment to the number of AFC in the control. The study of the effect of the preparations on expression of the Thy-I marker of T lymphocytes was carried out by the standard test of restoration of the sensitivity of the spleen cells of thymectomized mice to the inhibitory action of azothioprene (Az-RFC) [3]. The preparation containing a set of amino acids was obtained by an original method by extracting thymic tissue; individual amino acids were obtained by high-performance liquid chromatography on "Spherisorb-ODC 5 μ" columns, and Tactivin by the method in [5]. L-tyrosine also was obtained from "Sigma" (USA).

EXPERIMENTAL RESULTS

After injection of Tactivin into CBA mice, reacting strongly to SRBC, the value of IS-AFC varied depending on the dose of the preparation (Fig. 1A). Maximal values of IS-AFC, namely 8.8 and 7.9, were obtained with optimal doses of 1 and 100 μg per mouse. The least effective dose of the preparation (10 μg per mouse) induced stimulation of IgM-secreting AFC by 4.2 times compared with the control. These values, characterizing the immunostimulating activity of Tactivin, are much superior to the results obtained on an experimental model whose distinguishing feature was the predominant production of IgG-secreting AFC [2]. The optimal dose

Central Research Institute of Epidemiology, Ministry of Health of the USSR. Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Pokrovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 583-585, November, 1988. Original article submitted August 1, 1987.

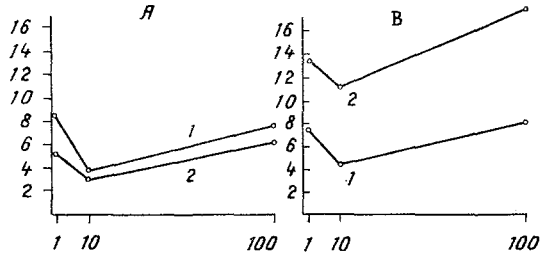

Fig. 1. Effect of immunologically active substances on index of stimulation of antibody-forming cells in CBA (A) and C57BL/6 (B) mice. Abscissa, dose of substances (in μg per mouse); ordinate, IS-AFC. 1) Tactivin, 2) L-tyrosine.

TABLE 1. Effect of Stimulation of AFC in Spleen of Mice Giving Opposite Response to SRBC after Injection of Tactivin or L-tyrosine (M ± m)

Preparation	Line	Number of AFC per 10^6 splenic karyocytes			
		1*	10*	100*	control
L-tyrosine	CBA	15,7±9,5	22,4±4,1	51,2±13,3	8,2±2,3
	C57BL/6	47,5±6,2	40,2±7,5	63,8±12,3	3,6±1,4
Tactivin	CBA	51,7±11,7	23,3±4,2	46,7±9,7	5,9±0,5
	C57BL/6	27,8±5,1	17,0±3,2	29,9±3,6	3,6±1,4

Legend. Preparations were injected intraperitoneally 1 h before immunization of mice with SRBC. Asterisk indicates doses of preparations in micrograms per mouse.

of the preparation for (CBA × C57BL)F_1 mice, reacting strongly to SRBC, caused an increase in the number of IgG-secreting AFC by not more than 2.5 times. Comparative analysis of the present results and data in the literature suggests that the formation of the IgM-secreting AFC population is more sensitive to the action of Tactivin than that of IgG antibody producers.

Comparison of IS-AFC for CBA and C57BL/6 mice receiving different doses of Tactivin showed that mice of both lines respond by intensification of their immune response to SRBC (Fig. 1A, B); values of IS-AFC, moreover, remained closely similar after injection of equivalent doses of the preparation. These data indicate that Tactivin increases IS-AFC in mice regardless of the opposite nature of their response to SRBC.

Comparison of the numbers of AFC per 10^6 splenic karyocytes in animals receiving Tactivin in doses of 1 and 100 μg per mouse showed that the highly reactive CBA line gave a much stronger immune response to SRBC than the weakly reacting C57BL/6 line (Table 1), i.e., in response to injection of the above-mentioned doses of Tactivin, both lines preserved the opposite nature of their response to xenogeneic erythrocytes. With a dose of Tactivin of 10 μg per mouse, differences in the number of AFC per 10^6 spleen cells in CBA and C57BL/6 mice were less marked. The results of this comparative analysis of IS-AFC and of the number of AFC in lines of mice with opposite directions of response show that against the background of the action of Tactivin, they preserve the determined values of strength of their immune response.

In the search for other regulators of production of IgM-secreting AFC, a set of amino acids were isolated from thymic tissue, in which glutamic and aspartic acids, histidine, tyrosine, glycine, lysine, and serine were present in the molar ratio of 8:2:2.5:2.7:2.3:2.3. This complex preparation and some of its individual components caused stimulation of AFC in C57BL/6 mice, but the most active amino acid was tyrosine.

On injection of L-tyrosine into CBA mice the value of IS-AFC was close to its values obtained for the corresponding doses of Tactivin (Fig. 1A), but in C57BL/6 mice receiving L-tyrosine, the value of IS-AFC showed a greater increase (Fig. 1B). Thus, L-tyrosine gives a more effective increase in the number of AFC in the weakly responding than in the strongly responding line of mice. After injection of L-tyrosine in doses of 1 and 100 μg per mouse,

the strength of the immune response to SRBC in both lines of mice was similar, but a dose of 10 μg per mouse led to the number of AFC in C57BL/6 mice being greater than in CBA mice (Table 1). Consequently, by contrast with Tactivin, L-tyrosine converts a genetically weakly responding line into a strongly responding line through an increase in the number of AFC in individuals responding weakly to SRBC.

This effect of L-tyrosine is probably exhibited only in response to injection of the free amino acids. Dipeptides Tyr—Leu and Leu—Tyr did not exhibit this specific action on C57BL/6 mice. Determination of activity of the test substances during stimulation of T-lymphocyte maturation showed that Tactivin was active in the Az—RFC test in doses from 3 μg/3 × 10^6 splenic karyocytes, and the amino-acid complex and L-tyrosine were inactive in doses of 1, 10, and 100 μg per mouse; these results suggest that there are differences in the mechanisms of stimulation of AFC production by these substances in mice responding in opposite directions.

LITERATURE CITED

1. R. V. Petrov, V. M. Khaitov, V. M. Man'ko, and A. A. Mikhailova, Monitoring and Control of the Immune Response [in Russian], Leningrad (1981), pp. 73-75.
2. R. V. Petrov, A. A. Mikhailova, L. A. Zakharova, et al., Immunologiya, No. 4, 42 (1982).
3. J.-F. Bach and M. Dardenne, Cell Immunol., 3, 11 (1972).
4. N. K. Jerne and A. A. Nordin, Science, 140, 3565 (1963).
5. J. M. Lopukhin, R. V. Petrov, V. J. Arion, et al., U. S. Patent 4377511 (1983).
6. R. H. Schwartz, Adv. Immunol., 38, 31 (1986).

PATTERNS OF PHENOTYPIC VARIATION IN DNA AUTOANTIBODY LEVELS IN HEALTHY PERSONS

Yu. V. Nesvizhskii, L. G. Sibiryakova, D. V. Korogodin, and A. A. Vorob'ev

UDC 612.124.017.1.06:/612.12.015.2:547.963.32/:612.118.221.2

KEY WORDS: HLA antigens; autoantibodies; DNA.

Autoantibodies to nucleic acids and, in particular, to DNA are among the generally accepted pathogenetic factors and immunodiagnostic parameters of systemic diseases of autoimmune nature [4]. Meanwhile the study of autoantibodies to DNA purely from the standpoint of pathology is to ignore a whole series of problems to do with the healthy, normal state. For instance, the absence of information on the character and degree of polymorphism of individual values of this parameter in the healthy human population not only reduces the diagnostic value of the test, but also restricts our ideas of the mechanisms of autoimmunity and the possibility of phenotypic correction of the autoimmune status.

The aim of this investigation was to study the pattern of distribution of levels of autoantibodies to native (n) and denatured (d) DNA and the role of the HLA system in determining their variation in healthy persons.

EXPERIMENTAL METHOD

Blood serum from 76 healthy blood donors with no clinical or laboratory evidence of autoimmune pathology was investigated. Group 1 consisted of 21 pairs of twins aged from 20 to 25 years (26 women and 16 men): 14 pairs were monozygotic and seven pairs were dizygotic. Zygosity was determined on the basis of anthropologic, anthropometric, dermatoglyphic, and serologic investigations [1]. Group 2 consisted of 34 unrelated test donors from the Laboratory of Immunogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, made up of 19 women and 15 men aged from 25 to 52 years (average age 32 years).

The level of autoantibodies (autoAB) of the M, G, and A isotypes of nDNA and dDNA was determined by enzyme immunoassay [10] using monospecific antiimmunoglobulin sera (Research Institute of Epidemiology and Microbiology, Gor'kii), and staphylococcal protein A labeled with

Institute of Immunology, Ministry of Health of the USSR. Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 585-587, November, 1988. Original article submitted October 16, 1987.