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

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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

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

TABLE 1. Relative Role of Genotype and Environment in Determination of Variation of Levels of AutoAB to nDNA and dDNA in Healthy Subjects

Antigen	Ig isotype	r_{mz}	r_{dz}	Genetic factors			Environmental factors		
				G*	Ga	Gd	E*	Ec	Ew
nDNA	M	0,659	0,432	0,454	0,454	0	0,546	0,205	0,341
	G	0,912	0,693	0,438	0,438	0	0,562	0,474	0,088
	A	0,596	0,323	0,546	0,546	0	0,454	0,050	0,404
dDNA	M	0,748	0,279	0,558	0,558	0	0,442	0,190	0,252
	G	0,524	0,421	0,206	0,206	0	0,794	0,318	0,476
	A	0,616	0,506	0,220	0,220	0	0,780	0,396	0,384

Legend. r_{mz}) Coefficient of correlation between twins of a pair for monozygotic twins; r_{dz}) the same, for dizygotic twins. *) Combined contribution.

TABLE 2. Association of Levels of AutoAB to nDNA and dDNA with HLA-Antigens

Autoantibodies	Ig isotype	HLA-antigens	r_{pb}	p
To nDNA				
High level	M	A3	0,346	<0,05
		B35	0,461	<0,01
	A	A3	0,535	<0,001
		B35	0,353	<0,05
Low level	M	A10	0,411	<0,01
		B17	0,489	<0,001
		B22	0,345	<0,05
	G	B17	0,390	<0,05
	A	B17	0,340	<0,05
To dDNA				
High level	M	A3	0,357	<0,05
		B35	0,394	<0,05
	G	B35	0,355	<0,05
		DP2	0,407	<0,05
	A	A3	0,495	<0,001
		B14	0,321	<0,05
		B35	0,350	<0,05
Low level	M	B17	0,347	<0,05
	G	B17	0,359	<0,05
		DP1	0,353	<0,05

Legend. r_{pb}) Coefficient of point-biserial correlation.

horseradish peroxidase (from "Sigma," USA). The results were estimated in optical density units (OU). nDNA was obtained from "Sigma" (USA), and dDNA was obtained by thermal denaturation of nDNA at 100°C for 10 min, followed by rapid cooling to 0°C.

Lymphocytes for titration of HLA antigens were isolated from peripheral blood by Böyum's method [9]. A lymphocyte population enriched with B cells was obtained with the aid of papainized sheep's red blood cells [13]. HLA antigens were determined by standard methods [8, 12], using a panel of antisera obtained from the National Institutes of Health (USA), the N. I. Pirogov Republican Scientific-Practical Institute of Emergency Medical Aid (Bulgaria), the Institute for the Study of Blood Diseases (France) and the Institute of Medical Genetics, Academy of Medical Sciences of the USSR. The following HLA antigens were typed: A1, A2, A3, A9, A10, A11, A28, A29, B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, 21, Bw22, B27, B35, B40, Bw41, Cw1, Cw2, Cw3, Cw4, DR1, DR2, DR3, DR4, DR5, DRw6, DR7, DRw8, DRw9. In the sample tested, the HLA antigens most frequently found were A2, A3, B7, B35, DR2, DR5, DRw8.

The results were subjected to statistical analysis. Analysis of the twins was based on calculation of the coefficient of correlation between the twins of a pair [5]. Component analysis of total phenotypic dispersion with estimation of additive (Ga) and dominant (Gd) genetic and of systematic (Ec) and random (Ew) environmental variances was carried out with the aid of an auxiliary matrix [3]. The presence of correlation between HLA antigens and tested autoAB levels was determined by calculating the coefficient of point-biserial correlation [2].

EXPERIMENTAL RESULTS

AutoAB of the M, G, and A isotypes of nDNA and dDNA were found in the blood serum of all the subjects tested. The distribution of individual values of the features studied was unimodal and had marked dispersion. The average level and its error for autoAB to nDNA corresponding to the immunoglobulin isotypes were 1.00 ± 0.022, 0.62 ± 0.12, and 0.86 ± 0.017 OU, and for autoAB to dDNA 0.87 ± 0.22, 0.60 ± 0.014, and 0.68 ± 0.016 OU respectively.

Analysis of twins revealed greater similarity in all variants examined between twins of monozygotic than of dizygotic pairs (Table 1). Hence it could be concluded that interindividual differences for levels of autoAB to nDNA and dDNA are determined by the cooperative action of genotype and environment. Further analysis of the total phenotypic dispersion of values of the features examined showed that genetic factors were represented only by an additive component; moreover, in the case of autoAB to nDNA, this fraction accounted for 44-55%. The inheritability of interindividual differences in autoAB levels of G and A isotypes of dDNA was 21-22%, whereas for IgM this parameter reached 56%. Environmental factors included both systematic and random components and their contributions to variation of the levels studied were of equal value, with the exception of IgG and IgA to nDNA.

In a comparison of the results of a determination of the levels of auto AB to nDNA and dDNA with the individual HLA phenotype, statistically significant correlations were established Table 2). Thus, association of an increased level of the investigated autoAB with the HLA antigens A3 and B35 was established (with the exceptions of antibodies of the G isotype). The low level of autoAB determinable, with the exception of IgA to dDNA, was observed primarily among carriers of HLA-B17. The statistical significance of the correlations noted was confirmed by the Mann-Whitney nonparametric method ($p < 0.05$). The other associations were not so systemic, and their level varied depending on the antigen structure and the Ig isotype.

Thus, in our work the major features of the distribution of levels of autoAB of the isotypes M, G, and A to nDNA and dDNA in a population of healthy people were revealed: unimodality and pronounced variability. An investigation of the etiology of the phenotypic lability of the characteristics noted, bringing in the twin model, showed that the property described is a product of the cooperative action of the genotype and the environment. The data obtained are confirmed to a definite degree by the results of an examination of the families of patients with systemic lupus erythematosus [6], which revealed a rather high degree of heritability of the levels of autoAB to nDNA.

The discovery that the contribution of genotype to variation in the levels examined on the structure of the antigen used in the work was a remarkable fact. For instance, conversion of nDNA into the denatured form was accompanied by a decrease in the contribution of genetic factors to the total phenotypic dispersion of IgG and IgA levels.

The scale of representation of genetic factors of additive variance suggests polygenic determination of interindividual differences in the levels of autoAB studied. Genes of the HLA locus may be among these genetic structures that are capable of determining interindividual differences between the features examined. For instance, it has been shown that for HLA-DR3 carrier, levels of circulating autoAB to a broad spectrum of autoantigens are characteristically elevated [7, 11]. We found correlation between levels of autoAB to nDNA and dDNA with individual HLA-antigens. The most stable association between the levels studied were observed with HLA-A3, B17, and B35.

Meanwhile, although they confirm the view that the HLA system is involved in the regulation of immune reactions, the results do not allow the mechanism of the effect of the concrete HLA phenotype on individual levels of autoAB to DNA to be correctly judged.

LITERATURE CITED

1. V. M. Gindilis and S. A. Finogenova, Genetika, 12, No. 8, 139 (1976).
2. M. G. Kendall and A. Stuart, The Advanced Theory of Statistics, Vol. 2, London (1979), p. 331.
3. E. T. Lil'in, V. M. Trubnikov, and M. M. Vanyukov, Introduction to Modern Pharmacogenetics [in Russian], Moscow (1984).
4. V. A. Nasonova and A. M. Poverennyi, Ter. Arkh., 39, No. 9, 38 (1967).
5. E. I. Sokolov, P. B. Gofman-Kadochnikov, and E. T. Lil'in, Outlines of Studies of Twins (in Clinical Medicine) [in Russian], Moscow (1980).
6. I. M. Adamashvili, O. V. Makarova, I. M. Kvachadze, et al., Immunol. Lett., 6, No. 1, 33 (1983).
7. J. N. Ambinder, N. Chiorazzi, A. Gobofsky, et al., Clin. Immunol. Immunopathol., 23, No. 2, 269 (1982).
8. G. Bodmer, P. Pickbourn, and S. Richards, Histocompatibility Testing 1977, Copenhagen (1977), pp. 35-84.
9. A. Böyum, Tissue Antigens, 4, No. 4, 269 (1974).
10. E. Engvall and P. Perlman, J. Immunol., 109, No. 1, 129 (1972).
11. T. J. Lawley, R. P. Hall, A. S. Fauci, et al., New Engl. J. Med., 304, No. 4, 185 (1981).
12. NAID Manual of Tissue Typing Techniques (1976-1977, Bethesda, Maryland (1977), pp. 22-24.
13. A. Wilson, D. G. Haegert, and R. R. A. Coombs, Clin. Exp. Immunol., 22, No. 1, 177 (1975).