VARIABILITY OF SENSITIVITY OF HEALTHY HUMAN LYMPHOCYTES TO ANTI-PROLIFERATIVE ACTION OF DEXAMETHASONE AND ANTIGENS OF THE HLA SYSTEM

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The ability of glucocorticoids to inhibit lymphoid cell proliferation evidently plays an important role in the mechanisms of the immunodepressive action of this group of preparations. Experiments on mice have shown a connection between the character of response of lymphoid cells to glucocorticoid treatment and antigens of the H-2 system [7, 9]. Attempts have also been undertaken to link individual sensitivity of the proliferative response of peripheral blood lymphocytes to corticosteroids with the HLA-phenotype in man. Associations of antigens B12 [3] and A10 [5] with higher sensitivity of lymphocytes to the antiproliferative action of glucocorticoids have been found. In the light of existing views on the role of antigens of the major histocompatibility complex in immune processes, the data given above are particularly interesting for, in particular, they offer good prospects for the study of the mechanisms of individual sensitivity to the immunodepressive action of glucocorticoid hormones.

In the investigation described below individual variability of sensitivity of human lymphocytes to the antiproliferative action of dexamethasone was studied and a search was made for the possible connection of this variability with the HLA phenotype.

EXPERIMENTAL METHOD

Mononuclear cells were isolated from heparinized peripheral blood of healthy blood donors of both sexes in a Ficoll—Verografin density gradient (ρ = 1.077) [4]. The cells

TABLE 1. Height of Proliferative Response of Lymphocytes and Corresponding ED_{50} Values for Donors with Different Types of Sensitivity

Resistant type			Intermediate type			Sensitive type		
No. of donor	response to PHA	inhibition of response to PHA	No. of donor	response to PHA	inhibition of response to PHA	No. of donor	response to PHA	inhibition of response to PHA
1	33 190	3,39	8	18 958	0,37	15	58 874	2,78
	59 874	14,45		41 357	2,02		200 787	4,81
2	45 707	7,52		47 572	1,28	16	61 084	0,82
	41 357	8,32		96 761	9,27		150 242	2,44
	134 592	26,92	9	194 853	17,58	17	29 144	0,62
3	169 397	69,34		88 433	2,72		47 099	1,42
	43 914	7,10						
			10	167 711	4,36	18	106 938	2,95
	57 526	4,06						
	81 117	34,25		62 944	7,94		125 492	4,36
4	24 343	3,72	11	43 045	1,47		96 492	5,28
	89 322	5,58		62 318	5,81	19	150 242	1,87
	206 902	109,60		90 219	5,71		69 564	3,89
5	99 708	10,30	12	202,805	8,28	20	80 822	0,83
	102 744	17,26		192 914	8,93		92 043	1,41
	79 221	11,04		135 944	24,21			
6	23 156	1,96	13	64 216	1,71	21	129 314	0,58
	169 397	12,88		98 716	6,59		29 144	1,02
7	35 596	7,24	14	150 242	7,74	22	126 754	4,59
	134 592	10,33		125 492	8,47		174 556	5,50
				147 267	10,81			

Legend. Response to PHA — data given in cpm after subtraction of background (spontaneous proliferation without PHA); inhibition of response to PHA — median active dexamethasone concentration ($ED_{50} \cdot 10^{-9}$ M).

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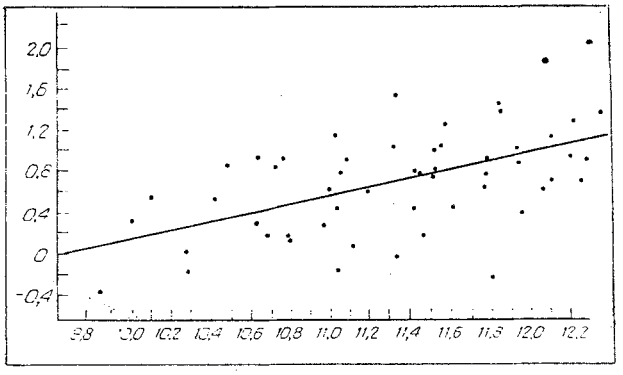

Fig. 1. Evaluation of correlation between sensitivity of lymphocytes to antiproliferative action of dexamethasone and height of response to PHA (theoretical regression line). Abscissa, ln (cpm); ordinate, log ED_{50}.

TABLE 2. Prevalence of Some HLA Antigens in Group of Resistant Donors Compared with Control

HLA antigen	Resistant donors	Control group	2P
B7	4 (7)	29 (200)	0,014
B12	4 (7)	31 (200)	0,018
DR2	5 (7)	43 (200)	0,017

Legend. Total number of donors in group given in parentheses.

were washed off twice and resuspended in medium RPMI 1640 ("Flow Laboratories," England) containing 10% inactivated embryonic calf serum of the same series ("Flow Laboratories"), $2 \cdot 10^{-3}$ M HEPES, 2 mM L-glutamine, $2.8 \cdot 10^{-6}$ M 2-mercaptoethanol, and 20 μg/ml of gentamicin. The cells were cultured in flat-bottomed 96-well panels ("Nunc," Denmark), $5 \cdot 10^4$ cells being added to each well in 200 μl of culture medium. The cells were stimulated by the addition of phytohemagglutinin (PHA-P, from "Difco," USA) in a concentration of 5 μg/ml. The proliferative response was inhibited by the use of dexamethasone ("Sigma," USA) in four different concentrations within the dose range from 10^{-9} to 10^{-6} M. Dexamethasone was not added to the control wells (they contained culture medium with and without PHA). The cells were incubated for 72 h at 37°C in a humid atmosphere containing 5% CO_2. ^{3}H-thymidine was added to each well 4-6 h before the end of culture in a dose of 40 kBq. After culture ceased cells were transferred to filters and their radioactivity estimated in a Mark III liquid scintillation counter ("Tracor Analytic," USA). The intensity of proliferation was expressed in cpm and the magnitude of the antiproliferative response in the form of the median active dose (ED_{50}), which was calculated by a variant of the probit method [1]. Each donor was tested from two to four times. Altogether 55 investigations were made of 22 blood donors.

HLA-antigens were typed by a standard microlymphocytotoxic test in the NIH modification [6], using a panel of 99 typing sera against 37 antigens of the A, B, C, and DR series. This panel included sera from the Institute of Medical Genetics, Academy of Medical Sciences of the USSR, the N. I. Pirogov Emergency Aid Institute (Sofia, Bulgaria), the National Institutes of Health (USA) and the firm of "Biotest" (West Germany). The control group consisted of 200 healthy, unrelated blood donors, collaborating with the Blood Transfusion Department of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The distribution of HLA antigens in donors of different groups was compared by Fisher's method without correction for the number of antigens tested [8]. When the results were analyzed, methods of correlation and regression analysis also were used [2].

EXPERIMENTAL RESULTS

During repeated tests of the same donors the height of the proliferative response when a constant dose of PHA was used varied significantly. The sensitivity of the peripheral

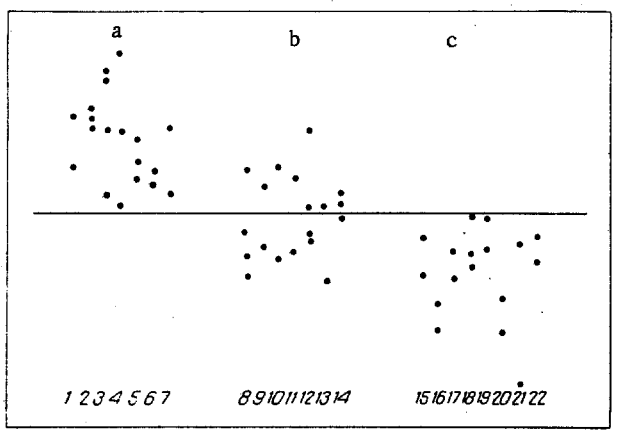

Fig. 2. Use of regression line to distribute donors among types of sensitivity to antiproliferative action of dexamethasone. a) Resistant type, b) intermediate type, c) sensitive type. Numbers indicate identification numbers of donors.

blood lymphocytes to the antiproliferative action of dexamethasone also varied significantly from one experiment to another. Nevertheless, comparison of data on the height of the proliferative response with data on sensitivity of the cells to the action of dexamethasone showed that the character of the results is not random, for direct positive correlation was found between these values ($r = 0.54$ with $df = 53$; $p < 0.01$). The coefficient of correlation, while indicating significant correlation between the parameters studied, is substantially less than unity, reflecting the appreciable scatter of the points around the regression line (Fig. 1). This scatter is evidence of the variability of individual sensitivity of the donors to the antiproliferative action of dexamethasone. The less this variability, the nearer to the regression line the experimental points lie. On the basis of these data the blood donors can be grouped according to the degree of sensitivity of their lymphocytes to the antiproliferative action of dexamethasone. To do this, using the regression line as a line of reference, all the points indicating determined values of ED_{50} have to be arranged in conventional units of distance from the regression line (Fig. 2). By means of the method described, all the donors studied were thus divided into three groups: a group of resistant donors (the results of all the determinations lie above the regression line), a group of sensitive donors (all points lie below the regression line), and a group of subjects with intermediate sensitivity, whose cells in some experiments showed moderate resistance and in others, moderate sensitivity (Table 1).

Comparison of the distribution of HLA antigens in these groups revealed a significant increase in the frequency of occurrence of B12 (57.1%, $2p = 0.018$), B7 (57.1%, $2p = 0.014$), and DR2 (71.4%, $2p = 0.017$) antigens in the group of resistant donors compared with their distribution in the control group (B12 — 15.5%; B7 — 14.5%; DR2 — 21.5%). In the other groups no significant deviation of the prevalence of HLA antigens could be found (Table 2).

The discovery of an association of HLA antigens with the type of sensitivity to the antiproliferative action of dexamethasone may be evidence in support of genetic determination of the differences discovered between individuals. The results also show that as a result of the presence of positive correlation between the sensitivity of human lymphocytes to the antiproliferative action of glucocorticoids and the height of the proliferative response of these cells to PHA (this last parameter is very variable) the value of ED_{50} cannot serve as the sole criterion that a given individual belongs to a particular sensitivity group. This type of assessment is possible by the method of regression analysis, by comparing the value of ED_{50} and the height of the proliferative response in the same experiment. This conclusion must be applied with caution to data [3, 5] obtained as a result of a single determination of the type of sensitivity of lymphocytes to the antiproliferative action of glucocorticoids, without taking into account the height of the response to the mitogen. The possibility cannot be ruled out that the discovery of two different associations in these investigations may be connected with this state of affairs rather than with population differences.

LITERATURE CITED

1. I. B. Pogozhev, Immunology and Prevention of Intestinal Infections [in Russian], Moscow (1962), pp. 191-216.
2. P. F. Rokitskii, Biological Statistics [in Russian], Minsk (1967).
3. B. Becker, D. H. Shin, and S. R. Waltman, Science, 194, 1427 (1976).
4. A. Böyum, Scand. J. Clin. Immunol., 21, Suppl. 96, 1 (1968).
5. R. P. Erickson, L. A. Heidel, J. J. Kapur, et al., Am. J. Hum. Genet., 37, 761 (1985).
6. J. G. Ray et al. (ed.), NIAID Manual of Tissue Typing Techniques, 1976-1977, Bethesda, Md. (1976), pp. 22-24.
7. M. Pla, J. Zakany, and I. Fachet, Folia Biol. (Prague), 21, 49 (1976).
8. G. Thomson, Theor. Popul. Biol., 20, 168 (1981).
9. M. L. Tyan, Immunogenetics, 8, 177 (1979).