of one large extremum, described by other workers previously [1, 2, 6, 7, 9, 11], by a more fractional titration we found several large maxima of roughly equal value. In this connection the presence of groups of cscDNA domains with different densities of supercoiling in nucleotides may be suggested. Thus the titration curves illustrated in Fig. 1b are evidence of topologic heterogeneity of DNA in human leukocyte nucleoids.

Differences in the leukocyte nucleoid titration curves for leukemia patients in Fig. 1b may prove useful for the diagnosis and analysis of the pathogenesis of leukemias and also, perhaps, of other types of pathology connected with disturbances of the genetic apparatus of cells.

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SENSITIVITY OF SPLEEN CELLS OF DIFFERENT STRAINS OF MICE TO THE ANTIPROLIFERATIVE ACTION OF ALKYLATING COMPOUNDS

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Differences in the sensitivity of mice of different lines to the immunodepressive action of alkylating agents depend on a number of genetically controlled factors. To analyze such a multifactorial system, its individual components must be distinguished so that the contribution of each of them to the general pattern observed in vivo can subsequently be determined.

The aims of the present investigation were to develop a quantitative method of assessment of the degree of sensitivity of lymphoid cells to the antiproliferative action of alkylating preparations and to study the effects of different alkylating compounds on proliferation of spleen cells of mice of different genotypes.

EXPERIMENTAL METHOD

Male mice weighing 20-24 g of the following inbred lines were used: BALB/cJLacSto, DBA/2 JSto, CC57Br/MVRap, C57Bl/6JSto. The animals were killed by cervical dislocation and the spleens were removed under sterile conditions, and transferred into a glass homogenizer to

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TABLE 1. Sensitivity of Spleen Cells of Different Lines of Mice to the Antiproliferative Action of Chlorbutin

Line of mice	Parameter	Dose of preparation, μg/ml					ED_{50}, μg/ml
		—	1	3	10	30	
BALB/c	Number of counts per min	88 876	127 405	79 386	17 343	1835	6,53
	Degree of inhibition	—	1,43	0,89	0,19	0,01	
DBA/2	Number of counts per min	175 614	127 165	115 184	17 806	4561	
	Degree of inhibition	—	0,72	0,65	0,09	0,02	2,73
CC57Br	Number of counts per min	126 025	151 567	100 040	20 304	3323	
	Degree of inhibition	—	1,20	0,79	0,17	0,02	5,56
C57Bl/6	Number of counts per min	191 566	155 323	136 743	25 095	3405	
	Degree of inhibition	—	0,81	0,71	0,13	0,01	3,36

Legend. After treatment with the preparation the cells were stimulated by conA. Degreee of inhibition — effect expressed in fractions of unity, taken as the control level. Number of counts per minute — data shown after subtraction of background value (incorporation of label by cells in absence of conA).

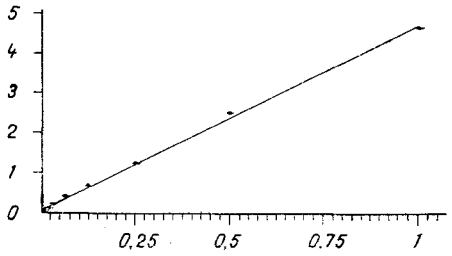

Fig. 1. Calibration graph plotted for bis(2-chloroethyl)amine hydrochloride (BCEH). Abscissa, concentration of BCEH ($\cdot 10^{-3}$ M); ordinate, optical density units.

obtain a cell suspension. The cell suspension was subjected to gravitational sedimentation for 5 min to remove fragments of stroma and washed with a large volume of cold culture medium, by single centrifugation at 400 g for 10 min. The cells were resuspended in medium RPMI-1640 (Flow Laboratories, Great Britain), containing 10% inactivated horse serum, $2 \cdot 10^{-3}$ M HEPES, $2.8 \cdot 10^{-6}$ M 2-mercaptoethanol, and 20 μg/ml of gentamicin. The following antiproliferative agents were used: chlorbutin without filler (from the "Oktyabr'" Leningrad Pharmaceutical Chemical Combine), a stable analog of an active metabolite of cyclophosphamide (CP) AstaZ7654 (the lysine salt of maphosphamide; from Asta-Werke, West Germany), a precursor of 4-hydroxycyclophosphamide, and active metabolites of CP (AMCP), the source of which was the serum of mice receiving an injection of cycophosphamide (cyclophosphan, from Saransk Medical Preparations Factory). To obtain the AMCP, male BALB/c mice were given an intraperitoneal injection of cyclophosphamide in a dose of 300 mg/kg 30 min before sacrifice. The concentrating of alkylating metabolites was determined by the NBP test [7] with certain modifications [4]. The results of the NBP test were expressed in moles, using bis(2-chloroethyl)amine hydrochloride as the standard.

The results of calibration are given in Fig. 1. To inhibit the proliferative response of the spleen cells various doses of the above-mentioned preparations in a volume of 100 μl were added to the wells in a flat-bottomed 96-well panel (Nunclon, from Nunc, Denmark). Next, 100 μl of a suspension of spleen cells in a concentration of $2 \cdot 10^6$ cells/ml was added to each well. The cells were incubated for 1 h in a moist atmosphere containing 5% CO_2, after which the panels were centrifuged at 4°C for 10 min at 400 g on a TJ-6 centrifuge (Beckman, USA). The supernatant was removed and the wells in the panel filled with fresh medium containing 5 μg/ml of phytohemagglutinin-P (PHA, from Difco, USA) or 40 μg/ml of concanavalin A (conA, from Calbiochem, USA). The cells were incubated under the same conditions for 72 h. Samples preincubated in culture medium not containing alkylating agents served as the control. Subsequent incubation of cells of the control groups was carried out both in the presence and in the absence of the mitogens. In all the experimental groups culture took place only in the presence of mitogens. In preliminary experiments the action of different doses of the preparations on the cells also was studied without addition of mitogens, so that later it was possible to use only concentrations which did not inhibit the

TABLE 2. Sensitivity of Spleen Cells of Different Lines of Mice to the Antiproliferative Action of Alkylating Agents

Line of mice	H-2 haplotype	Value of ED_{50} during exposure of spleen cells, treated with different preparations, to PHA and conA					
		AMCP, 10^{-5} M		maphosphamide, μg/ml		chlorbutin, μg/ml	
		PHA	conA	PHA	conA	PHA	conA
BALB/c	$H\text{-}2^d$	4,02		3,98	5,23	4,98	4,59
		3,03		4,65	4,51	4,64	4,71
		5,06	2,51	3,47	5,53	4,41	6,53
		2,75		4,86	3,56	4,67*	6,42
		3,61*		4,20*	4,47*		5,75*
DBA/2	$H\text{-}2^d$	1,12		1,45	1,88	2,41	2,50
		0,77		2,90	3,96	0,68	3,28
		1,33	1,77	2,01	2,93	2,68	2,73
		1,91		2,04*	2,79	1,64*	2,68
		1,22*			2,79*		2,78*
CC57Br	$H\text{-}2^b$	4,15		3,47	5,67	2,84	6,51
		2,34		3,14	5,87	2,26	5,56
		3,11	2,51	2,46	4,91	2,09	6,47
				4,33	5,55	3,00	7,77
		3,11*		3,28*	5,49*	2,52*	6,53*
C57Bl/6	$H\text{-}2^b$	0,85		0,78	3,76	2,12	3,42
		1,31		0,69	1,42	1,66	3,36
		1,45	1,33	1,95	1,66	2,76	4,42
		1,17*		1,05*	2,01	1,44	3,70*
					2,01*	1,93*	

Legend. Asterisk indicates geometric mean of individual determinations.

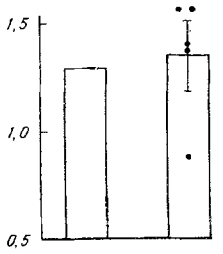

Fig. 2. Comparative sensitivity of DBA/2 mouse spleen cells to ACMP, determined individually (2) and from five mice (1). Ordinate — ED_{50} ($\times 10^{-5}$ M).

spontaneous proliferative response. ^{3}H-Thymidine was added 5 h before the end of culture in a dose of 40 kBq per well. At the end of culture, the cells were transferred by means of a "Cell Harvester" (Flow Laboratories, England) to filters whose radioactivity was counted in a Mark III liquid scintillation counter (Tracor Analytic, USA). The results were expressed as the median active dose (ED_{50}), which was calculated by a version of the probit method [6]. Values of ED_{50} obtained for mice of the different lines were compared by Wilcoxon's nonparametric test [2].

EXPERIMENTAL RESULTS

As was stated above, one aim of this investigation was to develop a quantitative method of estimating the sensitivity of lymphoid cells of animals with different genotypes to the antiproliferative action of drugs. For this purpose working concentrations of the agents were chosen, on the grounds that the majority of the doses used would give a quantitatively accountable effect, i.e., the percentage of inhibition of the proliferative response would be above 0 and below 100. The results of one experiment to compare the sensitivity of spleen cells on four lines of mice to the action of chlorbutin are given in Table 1. Under these circumstances, and in all experiments of this kind, a cell pool from three animals of the same strain was used. Preliminary experiments showed that the average results of individual determinations did not differ from those obtained on a pool (Fig. 2).

These experiments revealed two types of sensitivity of spleen cells from different lines of mice to the antiproliferative action of alkylating agents. For instance, cells of DBA/2 and C57BL/6 mice were distinguished by significantly greater sensitivity to all the compounds tested than cells of BALB/c and CC57Br mice (Table 2). By Wilcoxon's nonparametric test these differences were statistically significant in all cases when conA was used as the mitogen. In the case of stimulation by PHA, differences between cells of DBA/2 and CC57Br mice in their sensitivity to maphosphamide and chlorbutin, and also between cells of CC57Br and C57BL/6 mice in their sensitivity to chlorbutin were not statistically significant. Although the intensity of the proliferative response to mitogens differed in mice of different lines, no correlation could be found between the amplitude of the response and sensitivity to alkylating preparations (rank correlation coefficient $r = -0.3$). Differences found between the different lines were independent both of the mitogen used and on which alkylating agents were used. The type of sensitivity also was independent, evidently, of the H-2 haplotype. Thus lines of mice possessing the $H\text{-}2^d$ haplotype may be both sensitive and resistant. This applies also to lines with the $H\text{-}2^b$ haplotype (Table 2).

The results are in agreement with data showing that DBA/2 mice are more sensitive to the immunodepressive action of CP, thiophosphamide, and sarcolysin, than BALB/c mice [3, 5]. It can accordingly be postulated that interlinear differences in sensitivity to the antiproliferative action of alkylating agents, found in the present investigation, make a definite contribution to differences in the immunodepressive action of agents of this class. The results also demonstrate that strictly quantitative evaluation of individual densitivity to the antiproliferative action of therapeutic preparations is possible, so that there are real prospects for the introduction of this method into clinical practice.

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