ACTIVITY OF NORMAL KILLER CELLS AND THEIR SENSITIVITY TO INTERFERON IN OLD MICE

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Morphological and functional changes, connected with a progressive decline in ability to respond to foreign antigens and an increase in the frequency of autoimmune diseases, take place in the aging organism. As the effectiveness of the immune system weakens, the frequency of neoplastic diseases rises. Investigations [3, 7, 8] have shown that in mice during aging the activity of cells participating in antitumor resistance — so-called natural, or normal killer (NK) cells — decreases. Besides age, activity of NK is also affected by many other factors, including the genetic background and external environmental factors.

Interferon plays an important, perhaps key role in the regulation of NK activity [6]. A direct increase in NK activity in the mouse spleen was observed when the cells were treated *in vivo* and *in vitro* with interferon and substances inducing its synthesis [1, 4]. Analysis of the results of investigations to study the action of interferon on murine and human NK activity shows that interferon induces differentiation of NK precursor cells or activates (perhaps increases the activity of) the lytic mechanism of pre-existing mature NK [2, 9, 10]. Despite many investigations devoted to the study of this function of interferon, the problem is still insufficiently explained.

In the investigation described below the number of cells recognizing target cells (TC) sensitive to NK and causing their lysis in the spleen of old mice, and the ability of these cells to respond by increased activity to interferon and its inducer (a double-helical synthetic polynucleotide) were studied.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA, C57BL/6 (CBA × C57BL/6)F_1 hybrid, BALB/c, and A/Sn mice aged 2 months (young) and 12-20 months (old), obtained from the "Stolbovaya" and "Svetlye Gory" nurseries, Academy of Medical Sciences of the USSR. The double-stranded synthetic polynucleotide (poly-I:C) was injected intraperitoneally in a dose of 50 μg per mouse. Murine leukocytic interferon (α) was injected intraperitoneally in a dose of 4000 IU per young mouse and 6000 IU per old mouse. In the experiments *in vitro* interferon was used in doses of 300-1200 IU/ml. The animals were decapitated. A suspension of spleen cells was incubated with interferon for 1.5 h at 37°C. The cells were then washed three times.

To assess levels of DNA and RNA synthesis, spleen or thymus cells ($2 \cdot 10^6$) were incubated in medium RPMI-1640 with 10% embryonic calf serum and 1% glutamine in the presence of [^{3}H]-thymidine (5 μCi/ml, specific activity 23 Ci/mmole) or [^{3}H]uridine (5 μCi/ml, specific activity 26 Ci/mmole) for 1 h at 37°C. Radioactivity incorporated into the acid-insoluble fraction was estimated by means of a Packard Tricarb scintillation counter.

Natural cytotoxic activity of the spleen cells was determined in the test based on liberation of ^{51}Cr from labeled TC, for which cells of a YAC-1 mouse lymphoma, transplanted *in vitro*, were used. The TM ($5 \cdot 10^6$-$10 \cdot 10^6$) were incubated with 100 μCi $Na_2{}^{51}CrO_4$ (specific activity 1 mCi/mmole, Amersham Corporation, England) for 60 min at 37°C. TM ($2 \cdot 10^4$ cells in 0.1 ml medium) were introduced into wells of round-bottomed plates with an equal volume of effector cells (EC) in the ratio of 1:100, 1:50, or 1:25. The cells were incubated for 4 h at

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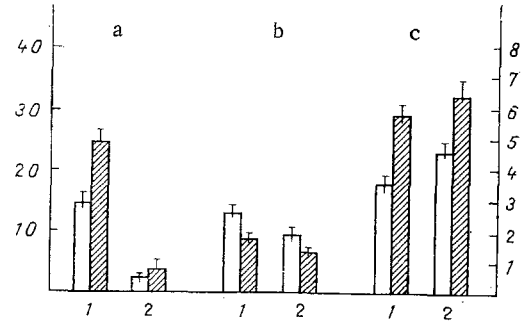

Fig. 1. Effect of poly-I:C on cytotoxic activity of NK (a) and incorporation of [^{3}H]thymidine (b) and [^{3}H]uridine (c) by spleen cells of young and old mice. Ordinate: on left, CI (in %); right, number of counts per minute per $2 \cdot 10^6$ cells ($\cdot 10^{-4}$). 1) Young mice; 2) old mice. Unshaded columns, control; shaded, experiment.

TABLE 1. Level of Natural Cytotoxic Activity and Number of EC in Spleen of Young and Old Mice

Line and age of mice	CI, % (EC : TC = 50 : 1)	Number of conjugates, %	Number of "effective" conjugates, %	EC, %
CBA:				
young	15,4 ± 1,2	4,58 ± 0,36	35,0 ± 2,34	1,75 ± 0,42
old	2,1 ± 0,5	2,72 ± 0,56	22,5 ± 4,4	0,59 ± 0,10
(CBA×C57BL) F_1:				
young	16,4 ± 1,5	6,01 ± 0,45	20,2 ± 0,5	1,2 ± 0,15
old	9,6 ± 1,3	2,06 ± 0,30	10,2 ± 0,7	0,2 ± 0,04
BALB/c:				
young	5,3 ± 0,8	4,85 ± 0,40	24,4 ± 0,6	1,16 ± 0,10
old	1,6 ± 0,4	2,98 ± 0,20	13,65 ± 1,20	0,40 ± 0,05
C57BL/6:				
young	13,6 ± 0,7	4,8 ± 0,34	22,3 ± 0,8	1,07 ± 0,10
old	5,7 ± 0,4	3,46 ± 0,28	16,6 ± 1,2	0,57 ± 0,08
A/Sn:				
young	3,8 ± 0,2	4,48 ± 0,32	23,0 ± 1,5	1,03 ± 0,20
old	2,0 ± 0,1	2,4 ± 0,2	16,3 ± 0,9	0,39 ± 0,12

TABLE 2. Effect of Interferon on Level of Natural Cytotoxic Activity and Number of EC in Spleen of Young and Old Mice

CBA mice	Administration of interferon	CI, % (EC : TC = 50 : 1)	Number of conjugates, %	Number of "effective" conjugates, %	EC, %
Young	—	15,4 ± 1,2	4,58 ± 0,36	45,0 ± 2,24	1,75 ± 0,42
	in vivo	30,2 ± 2,8	7,34 ± 0,09	47,2 ± 2,8	3,82 ± 0,38
	in vitro	21,5 ± 2,3	6,60 ± 1,07	52,2 ± 3,1	2,95 ± 0,09
Old	—	2,1 ± 0,5	2,72 ± 0,56	22,5 ± 4,4	0,59 ± 0,10
	in vivo	2,7 ± 0,6	4,10 ± 0,95	22,5 ± 2,5	1,02 ± 0,20
	in vitro	2,6 ± 0,4	3,24 ± 0,36	26,8 ± 2,5	0,93 ± 0,20

37°C. The plates were then centrifuged at 200g for 3 min. Radioactivity of 0.1 ml of supernatant was measured on a "Rack-Gamma II" Gamma counter. The cytotoxic index (CI) was calculated by the equation:

$$CI = \frac{\text{number of counts (experiment} - \text{spontaneous yield)}}{\text{number of counts (maximal yield} - \text{spontaneous yield)}} \times 100\%.$$

To obtain CI—lymphocyte conjugates (pairs) the method in [5] was used. Spleen cells were mixed with TC in the ratio of 1:1 or 5:1. The cell mixture was incubated at 37°C for 5 min and centrifuged at 200g for 5 min. The residue was resuspended and mixed with an equal volume of 1% solution of melted agarose, cooled to 42°C. The mixture was quickly transferred to plastic petri dishes. Medium was added to the dishes 1-2 min after gel formation and they were transferred into an incubator. After incubation for 3 h at 37°C, 0.1% trypan blue solution was added to the dishes for 5 min. They were washed twice with physiological saline and fixed with 1% formalin solution for 30 min. After washing the preparations were counterstained with methyl green and pyronine. The percentage of cells which formed junctions with TC, and the percentage of "effective" junctions of conjugates in which EC caused death of TC, was calculated.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Results of determination of natural cytotoxic activity in the ^{51}Cr liberation test and on isolated cell conjugates in agarose gel are given in Table 1. The level of cytotoxicity of the spleen cells of old mice (age 12-20 months) of all lines studied was considerably lower than for young mice (2 months). The number of cells binding TC was reduced in the old mice by 33-67%. The number of "effective" conjugates, reflecting the proportion of cells producing lysis of bound TC, was 40-100% less in old mice. The number of cells capable of producing lysis of TC in the spleen of the old mice was thus three to five times less than in young animals of the same line.

Injection of the interferon inducer poly-I:C into young mice caused a marked increase in the cytotoxicity of the spleen cells, whereas the increase in cytotoxic activity of spleen cells of old mice was not significant (Fig. 1). Poly-I:C inhibited incorporation of [^{3}H]-thymidine by spleen and thymus cells. Incorporation of [^{3}H]uridine by spleen cells was increased, whereas that by thymus cells was reduced. These changes were similar in type in young and old mice.

When interferon was used, the results in Table 2 were obtained. Injection of interferon *in vivo* into young mice or treatment of spleen cells *in vitro* caused a marked increase in the cytotoxicity index in the ^{51}Cr liberation test and an increase in the number of both cells binding TC and cells producing lysis of bound TC. Similar treatment with interferon of cells from old mice led to some increase in the parameters of cytotoxicity of these cells, but this increase was not statistically significant.

The considerable decrease in the level of natural cytotoxicity and in the number of cells capable of binding TC sensitive to NK, and causing their lysis, occurred in old mice of the lines studied, whether with high [CBA, C57BL/6, (CBA × C57BL/6)F_1] or with a lower level of NK activity (A/Sn and BALB/c). The decrease in the number of cells binding TC could be explained by a decrease in the number of cells possessing receptor structures for NK, or a decrease in the density of the latter on the surface of EC. The lower percentage of effective junctions may be evidence of a change in the number of regulatory cells or mediators, inducing maturation of NK precursors (capable of recognizing TC, but not causing their lysis) or activating the lytic mechanism in mature TC, in the old animals.

To decide whether the considerable reduction in cytotoxic activity and in the number of cells capable of exerting a cytotoxic action in the spleen of the old mice is connected with lowering of the level or total absence of interferon synthesis, due to a deficiency of endogenous or exogenous interferon inducers, poly-I:C was injected into the animals. Injection of this highly effective inducer caused a significant increase in the level of cytotoxic activity of NK in the young mice, but had no such effect on the old mice. Consequently, the low level of NK activity in the old animals could not depend purely on a possible deficiency of interferon inducers. Poly-I:C inhibited DNA synthesis equally, as could be deduced from a fall in [^{3}H]thymidine incorporation in spleen and thymus cells of both old and young animals. However, the level of RNA synthesis rose in the spleen cells, but fell in the thymus cells in both young and old mice. This was possibly connected with a change in the cell composition in these organs as a result of migration of thymus cells into the spleen, for the number of cells in the thymus fell by 30%, whereas in the spleen it increased after injection of the interferon inducer.* These changes were similar in young and old mice.

The absence of activation of NK in old mice in response to injection of the interferon inducer may perhaps be due to a defect in the cell population treated with interferon. In that case, injection of exogenous interferon ought to lead to an increase in NK activity provided that the state of the natural killer system is normal. However, injection of leukocytic interferon caused a considerable rise both in the level of NK activity and in the number of cells capable of binding and killing TC, in young mice, but without causing any significant increase in EC activity in old animals. Similar results were obtained when spleen cells were stimulated with interferon *in vitro*.

The decrease in the cytotoxic activity of NK in old mice and the inability of NK to respond to interferon by an increase in NK activity are thus evidently associated not only with possible defects in auxiliary cells, but also with changes in the EC population itself.

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INDUCTION OF T CELLS PRODUCING MACROPHAGE MIGRATION INHIBITION FACTOR BY MUTANT H-2 ANTIGENS *IN VIVO*

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The main function of the immune system is to protect the organism against all that is biologically foreign, whether entering from outside or arising inside the organism as a result of a change in its own cells produced by a virus, carcinogen, or mutation [1]. Mutant lines of Hz1 ($H\text{-}2^{bm1}$) and M505 ($H\text{-}2^{bm3}$) mice differ from the original C57BL/6 ($H\text{-}2^{d}$) line by replacement of one or two amino acid residues in the molecule of the transplantation antigen [8], and the reaction of C57BL/6 lymphocytes to Hz1 ($H\text{-}2^{bm1}$) and M505 cells can be used as a model for the detection of weak changes in the organism's own antigens at different stages of carcinogenesis.

It was shown previously that mutant bm1 and bm3 antigens differ serologically only slightly [8], but they effectively induce antigen-specific proliferation of T cells and generation of T killer cells in mixed lymphocyte culture (MLC) *in vitro* [8, 9]. The authors have shown that by the second or third day, i.e., before proliferation develops and T killer cells appear, T cells producing macrophage migration inhibition factor (MIF) are formed in MLC of mutant and the original lines [6].

In the investigation described below, a technique devised by the authors themselves was used to induce MIF producers in the H-2 system by intravenous immunization [4] and to study the ability of MIF-producing T cells to respond to mutant antigens $H\text{-}2^{bm1}$ and $H\text{-}2^{bm3}$ by immunization *in vivo*.

EXPERIMENTAL METHOD

The genetic characteristics of inbred lines of mice used in the investigation are indicated in Table 1. C57BL/6 mice (abbreviated to B6, $H\text{-}2^{b}$) were immunized by a single intra-

TABLE 1. Genetic Characteristics of Lines of Mice Used for Immunization *in Vivo* against Mutant and Normal Transplantation Antigens

Line of mice	Abbreviated name of line	H-2 haplotype	Regions of H-2			Genetic basis of line
			K	I	D	
C57BL/6	B6	b	b	b	b	B6
B6.C-H(z1)Y	Hz1	bm1	bm1	b	b	B6
B6.M505Y	M505	bm3	bm3	b	b	B6
B10.D2	D2	d	d	d	d	B10

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