Under these circumstances the number of T-TSL, which were mainly suppressor T cells [8] was reduced: 30 ± 3.2% after treatment of the cells with patients' serum and 44 ± 3.5% on incubation of lymphocytes with normal serum.

The results are evidence that the effect of antilymphocytic antibodies obtained from patients with various diseases is aimed at the suppressor T-cell population, whose functional activity is significantly depressed under the influence of antibodies, in agreement with the data in the literature on the antisuppressor orientation of autolymphocytic antibodies in systematic lupus erythematosus [6] and rheumatoid arthritis [10].

The appearance of antibodies against T lymphocytes in various diseases, described by the present writers and others [9], is not pathognomonic for a particular disease but is a general phenomenon, reflecting the state of the regulatory mechanisms of homeostasis as a whole.

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ROLE OF G_0- AND G_1-SPLENOCYTES AND OF ANTIGEN-BINDING LYMPHOCYTES PRODUCING ANTIGEN-DEPENDENT NONSPECIFIC IMMUNOLOBULINS

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KEY WORDS: antigen-bInding cells; splenocytes; antigen-dependent nonspecific immunoglobulins.

Induction of the specific immune response and the appearance of antibody-forming cells (AFC) are accompanied both *in vivo* and *in vitro* by an increase in the number of nonspecific immunoglobulin-forming cells (NIGFC) [4, 11]. In particular, we do not know whether antigen-dependent NIGFC (adNIGFC) are formed from resting precursor cells (G_0 cells) or from immunoglobulin-producing cells already present in the body (IGFC). These "background" IGFC in the mouse spleen account for usually 0.1-0.2% of the total number of cells. Most of them are B cells, in the G_1 phase of the cell cycle, i.e., they are blast cells. In addition, the writers showed previously that antigen-binding cells (ABC) participate in the formation of adNIGFC [1]. To elucidate the role of "background" IGFC in the formation of adNIGFC, in the investigation described below NIGFC formation was studied in a suspension of normal splenocytes and of splenocytes from which G_1 cells had been removed. It was also interesting to determine how removal of ABC affects the ability of splenocytes, after exhaustion of all G_1 cells, to form adNIGFC.

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EXPERIMENTAL METHOD

Female CBA mice weighing 16-18 g, obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, were used. The animals were lethally irradiated in a dose of 900-1100 rads and were restored with syngeneic splenocytes, injected intravenously in a dose of $(25\text{-}50)\cdot10^6$ per mouse simultaneously with antigen—sheep's red blood cells (SRBC) in a dose of $(125\text{-}250)\cdot10^6$ per mouse — or without antigen. Unrestored irradiated mice were used as the control. On the 5th day after transfer of the cells the number of IgM-AFC [6] and of IGFC [7] in the spleens of individual mice was determined. The number of MIGFC was calculated as the difference between the number of IGFC and the number of AFC per 10^6 splenocytes. The results are presented in the form M ± m.

Both normal splenocytes and cells fractionated in a Percoll density gradient into G_0 and G_1 cells [10] were used for adoptive transfer. To fractionate the splentocytes ($200\cdot10^6$ in 0.5 ml of medium) they were layered on a three-step Percoll gradient with densities of 1.03 g/cm^3 (5 ml), 1.072-1.074 g/cm (30 ml), and 1.09 g/cm^3 (5 ml) and centrifuged at 2000g for 20 min at 20°C. In the majority of experiments the fractionation procedure was undertaken twice. After centrifugation once or twice in a density gradient, cells which were not present in the layers 1.072-1.074 g/cm^3 (G_1) and 1.09 g/cm^3 (G_0) were washed 5 times with Eagle's medium, and their separation into G_0- and G_1-populations was verified by determining the number of IGFC in each fraction. Fractions enriched with G_0- and G_1-lymphocytes respectively were injected into irradiated recipients in a dose of $(25\text{-}50)\cdot10^6$ cells together with or without $(125\text{-}250)\cdot10^6$ SRBC.

To obtain splenocytes containing neither G_1 cells nor ABC, from a population enriched with G_0 cells, rosette-forming cells specific for SRBC were removed [1] by centrifugation in a Percoll gradient. As a result, additional removal of G_1 cells and also removal of ABC took place.

EXPERIMENTAL RESULTS

On average 9 ± 2 AFC to SRBC and 2342 ± 321 NIGFC per 10^6 cells were found in the spleen of the irradiated recipient mice (Table 1). Restoration of the lethally irradiated animals with syngeneic normal splenocytes itself led to an increase in the number of AFC and NIGFC in the spleen on the 5th day. Immunization of the restored mice with SRBC, even against the background of this increase, induced a sharp increase in the number of both AFC and NIGFC in these animals (Table 1). Thus in the adoptive transfer system, just as previously in experiments *in vivo* [4] and *in vitro* [1, 11], together with a specific immune response, a marked nonspecific immune response also was observed. The increase in the number of adNIGFC under these circumstances amounted to 22,954 NIGFC per 10^6 cells (Table 1).

To study the role of G_0 and G_1 cells in adNIGFC formation, irradiated recipients were restored with suspensions of splenocytes after removal of G_1 or G_0 cells. Twofold removal of G_1 cells from the suspension caused a reduction in the number of "background" IGFC in it from 0.22 to 0.014%. Transfer of these suspensions, deprived of G_1 (i.e., resting cells) or of G_0 lymphocytes into irradiated recipients led to the appearance of a rather smaller number of ARC in their spleens (38 ± 17 per 10^6 cells) and of a much smaller number of NIGFC (3983 ± 522 per 10^6 cells) than transfer of normal splenocytes (compare groups 2 and 4 in Table 1). This indicates that NIGFC formation, observed during restoration which normal splenocytes, is due mainly to G_1 cells. Injection of antigen into animals restored with G_0 cells caused the formation of 691 ± 119 AFC to SRBC and of 16,337 ± 3479 IGFC per 10^6 cells (see Table 1, group 5), i.e., both the formation of specific AFC and an increase in the number of adNIGFC were at about only half the level as during transfer of normal spleen cells (see Table 1, groups 3 and 5). This is evidence that the formation of AFC and adNIGFC is linked at least partially with G_1 cells. Participation of the latter was discovered in direct experiments with transfer of G cells. It was shown that on the 5th day the recipients' spleens contained 417 ± 59 AFC and 62,020 ± 5192 NIGFC. Injection of antigen increased both of these values highly insignificantly.

An antigen-dependent increase in the number of NIGFC thus cannot be detected as a result of transfer of G_1 cells (by contrast with what is observed with transfer of G_0 cells). These data confirmed our hypothesis that the appearance of the majority of NIGFC during restoration of irradiated mice with normal splenocytes (in the absence of antigen) is due to G_1 cells which, in the absence of exogenous antigen, can differentiate into IGFC (we know that the number of blast cells in a suspension of normal mouse splenocytes is about 5-9%).

TABLE 1. Induction of Immune Response in Irradiated Recipients, Restored by Various Subpopulations of Syngeneic Splenocytes (M ± m)

No. of group	Cells injected	Number of AFC and NIGFC per 10^6 cells		Increase in number of adNIGFC	Inhibition of increase in number of adNIGFC, %
		AFC to SRBC	NIGFC		
1 (n=36)	—	9± 2	2 342±321	—	—
2 (n=36)	Normal splenocytes	49±13	10 746±1 078	—	—
3 (n=36)	Normal splenocytes + SRBC	1 240±145	33 700±3 417	22954	0
4 (n=23)	G_0 lymphocytes	38±17	3 983±522	—	—
5 (n=23)	G_0 lymphocytes + SRBC	691±119	16 337±3 479	12354	46,2
6 (n=7)	G_1 lymphocytes	417±59	62 020±5 192	—	—
7 (n=8)	G_1 lymphocytes + SRBC	770±83	70 983±7 567	—	—
8 (n=11)	G_0, not containing ABC	98±14	3 616±880	—	—
9 (n=11)	G_0, not containing ABC + SRBC	117±18	5 388±1 498	1772	92,3

Note. n) Number of animals in group.

Since the writers showed previously in experiments *in vitro* [1] that ABC participate in adNIGFC formation, it was interesting to study how simultaneous removal of G_1 cells and ABC affects this process. During transfer of a cell suspension containing neither blast cells nor ABC specific for SRBC, we observed the appearance of 98 ± 14 AFC and 3616 ± 880 NIGFC in the recipients' spleen (Table 1, group 8). Injection of the antigen induced virtually no immune response in them. The number of AFC to SRBC did not exceed 117 ± 18 per 10^6 cells; the increase in the number of adNIGFC also was not significant (Table 1, group 9).

Thus removal of ABC from the G_0-cell population led to a decrease in NIGFC formation by 67% and to inhibition of the increase in the number of adNIGFC by 92.3%. Since the method of rosette formation which we used does not enable T rosettes to be obtained [5], it can be tentatively suggested that the inhibition of NIGFC formation observed in the case of transfer of G_0 splenocytes after removal of ABC from them is connected mainly with removal of ABC of B-cell origin. Hence it follows that adNIGFC formed from ABC ought to be cells which secrete either low-affinity or "defective" antibodies. Since it was shown previously that antigen-dependent nonspecific immunoglobulins are not low-affinity antibodies [12], we are left with the second possibility. The formation of "defective" antibodies may be due to fine differences in the structure of the antigen-specific receptors of ABC-precursors of AFC and NIGFC, which the writers postulated previously [1]. A certain number of adNIGFC is undoubtedly formed from B blast cells that produce antibodies to various nonhomologous antigens. It is interesting to note that these cells proliferate and differentiate under the influence of nonspecific blockers, released from activated T cells, and in the absence of specific antigen [3, 8]. Our data on G_1-cell transfer confirm this hypothesis.

We can thus propose the following simplified scheme of the mechanism of adNIGFC formation during antigenic stimulation *in vivo*. Antigen entering the body binds with specific G_0-B cells (G_0B-ABC) and helper T cells, which recognize processed antigen on the surface of the accessory cells. After binding antigen the G_0C-ABC are converted into G_1-B cells (G_1B-ABC) [9], capable of receiving signals from helper T cells [13]. In turn, activation of specific helper T cells for this antigen leads to the production of several T factors [2], which "trigger" not only "their own" but also "foreign" B cells, which are already in the G_1 stage (blasts). As a result the G_1B-ABC differentiate into producers both of antibodies specific for the injected antigen (AFC) and of "defective" antibodies (NIGFC). Some G_1B lymphocytes of other specificities may also be triggered by nonspecific T factors and they also differentiate into NIGFC. The latter probably produce antibodies to certain extraneous antigens.

The sources of adNIGFC are thus both G_0B cells that specifically bind the injected antigen and G_1B lymphocytes pre-existing in the body.

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PROPHYLACTIC EFFECTS OF SODIUM AND LITHIUM HYDROXYBUTYRATE DURING STRESS-INDUCED DEPRESSION OF NORMAL KILLER CELL ACTIVITY IN MICE

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Natural or normal killer (NK) cells play an important role in the maintenance of tissue homeostasis [7], in antitumor and anti-infectious protection [10, 11], and in regulation of a number of important biological processes [9, 11]. NK cell activity can be changed as a result of injury to various parts of the brain and, in particular, the hypothalamus [8], and under the influence of hormones, especially glucocorticoids [6]. It was shown previously that NK cell activity is significantly inhibited during emotional-painful [4] and immobilization stress [5], and that this inhibition can be abolished prophylactically by the cyclic derivative of delta-sleep peptide [3]. A marked prophylactic effect also is produced by sodium hydroxybutyrate (Na-OHBA) [3]. The high antistressor efficacy of a new preparation, namely lithium hydroxybutyrate (Li-OHBA), used for the treatment of various nervous and mental disorders, has recently been established [1, 2]. The aim of this investigation was to compare the prophylactic effect of these two substances — Na-OHBA and Li-OHBA — against stress-induced depression of NK cell activity.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 12 weeks. Immobilization stress for 6 h was induced by fixing the animals by their limbs in the supine position. The compounds for testing were given as a single intraperitoneal injection, 30 min before the beginning of exposure to stress, in the following doses: Na-OHBA 50, 100, and 200 mg/kg, Li-OHBA 50 mg/kg, and lithium chloride (LiCl) 10 mg/kg. The animals were divided into five groups: 1) mice receiving an injection of physiological saline (control); 2) stress (immobilization for 6 h); 3) Na-OHBA + stress; 4) Li-OHBA + stress; 5) LiCl + stress.

The animals were decapitated 24 h after the end of exposure to stress. A suspension of splenocytes, obtained by gentle mechanical destruction of the spleen in a glass homogenizer with Teflon pestle, followed by filtration of the cells through a nylon filter and by washing twice in Eagle's medium, was used as the source of NK cells. The cell concentration was adjusted to $20 \cdot 10^6$/ml and the cells were transferred to culture medium (RPMI 1640 medium with 10% embryonic calf serum and 1% glutamine). NK cell activity was determined by the test based on release of ^{51}Cr from labeled YAC-1 target cells (TC; a T-cell mouse lymphoma, maintained by subculture *in vitro*). For this purpose, to $5 \cdot 10^6$ YAC-1 cells in a volume of 1 ml of culture fluid was added 100 μCi of $Na_2{}^{51}CrO_4$ (specific activity 300-500 mCi/mg, from Amersham International, England) and incubated on a water bath at 37°C for 60 min. The TC

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