EFFECT OF SYNTHETIC COMPOUND B 58 ON NATURAL KILLER AND CYTOSTATIC CELL ACTIVITY IN THE MOUSE SPLEEN

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In recent years research has been directed increasingly toward the cellular mechanisms of antitumor resistance. Together with specific effectors of humoral and cellular immunity, certain categories of cells exhibiting spontaneous or lymphokine-induced cytotoxicity toward tumor cells also have been studied. The population of natural killer cells (NKC) has been studied the most [9, 10]. An important role in antitumor resistance may evidently be played by cells exhibiting cytostatic activity [5, 6].

The aim of this investigation was to study the effect of compound B 58, a synthetic interferon inducer, on activity of NKC and cytostatic effectors in the mouse spleen.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA, (CBA × C57BL/6)F_1, A/Sn, and BALB/c mice aged 8-10 weeks. Compound B 58 was injected intraperitoneally into the animals in doses of 10 and 100 μg per mouse in 0.5 ml of Eagle's medium. The mice were killed at different times after injection of the compound. NKC activity in the spleen was determined in the 4-hour microtoxicity test against VAC-1 target cells, labeled with ^{51}Cr [1]. To determine activity of the cytostatic cells, our modification of Gadiot's test [6], based on recording inhibition of DNA synthesis in P 815 target cells, was used. Effectors and targets were incubated for 4 h in 96-well panels in medium RPMI-1640 with 10% embryonic calf serum in the ratio of 20:1. Next, 1 μCi of ^3H-thymidine (specific radioactivity 5 Ci/mmole) was added to each well and the cells were incubated for a further 4 h, after which they were transferred to filters. Incorporation of ^3H-thymidine into the cells was estimated on a β-counter. The cytostatic index (CI) was calculated by the equation:

$$CI = \frac{1 - \text{cpm in experiment} - \text{cpm in wells with effector cells.}}{\text{cpm in wells with target cells}}$$

The serum interferon level was determined in a pool of sera from 5-10 animals, using L cells and vesicular stomatitis virus, strain Indiana, in a dose of 100 TCD_{50} as the indicator virus [3, 4]. To determine the type of interferon obtained the preparations were heated (60°C, 30 min) or exposed at pH 2.5 for 24 h, after which its antiviral activity was determined.

Production of interleukin 2 was induced in a population of spleen cells ($5 \cdot 10^6$/ml) by compound B 58 or by concanavalin A (con A) in medium RPMI-1640 with 5% embryonic calf serum, 2 mM glutamine, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, and 50 μg/ml of gentamicin. Interleukin-2 activity was estimated from its ability to maintain growth of T blast cells [2].

EXPERIMENTAL RESULTS

Compound B 58 induced an increase in NKC activity in the spleen of CBA mice, which are characterized by an initially high level of cytotoxic activity against NKC-sensitive VAC-1 targets. In A/Sn mice, with an initially low level of NKC cytolysis, compound B 58 did not increase NKC activity (Fig. 1a). This may be due to the relatively low inducibility of in-

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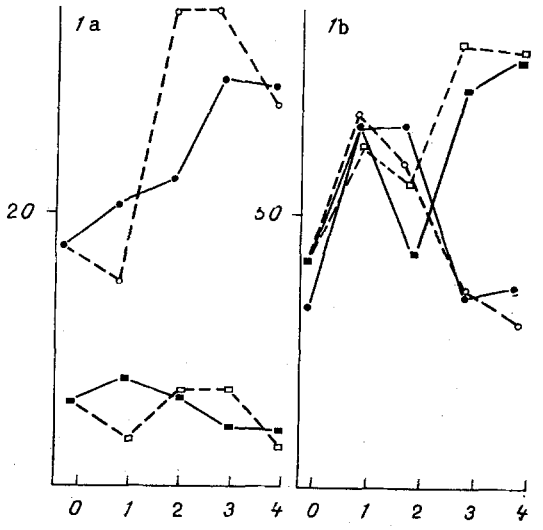

Fig. 1. Effect of compound B 58 on activity of NKC and cytostatic spleen cells of CBA and A/Sn mice. 1a) Activity of NKC against VAC-1 targets; 1b) activity of cytostatic cells against P 815 targets. Abscissa, time after injection of B 58 (in days); ordinate: a) cytolysis (in %); b) cytostasis (in %). Circles) CBA mice; rectangles) A/Sn mice; filled symbols – 10 μg per mouse; empty symbols – 100 μg per mouse.

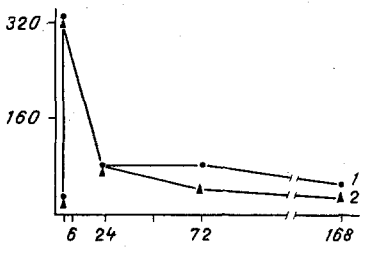

Fig. 2

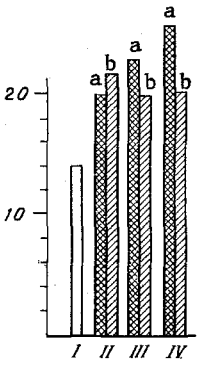

Fig. 3

Fig. 2. Serum interferon level in CBA mice after intraperitoneal injection of compound B 58. Abscissa, time after injection of B 58 (in h); ordinate, interferon titers. 1) 100 μg, 2) 10 μg.

Fig. 3. Effect of compound B 58, polyI:C, and α-interferon on NKC activity of (CBA × C57BL/6)F_1 mice against VAC-1 targets. Abscissa, versions of experiment; ordinate, cytolysis (in lytic units). I) Control; II) B 58; III) polyI:C; IV) α-interferon. a) NKC activity after 24 h, b) the same after 48 h.

terferon production in mice of this strain [9]. Maximal NKC activity in CBA mice was observed on the 2nd-4th day after injection of B 58.

Compound B 58 sharply increased the cytostatic activity of splenocytes against P 815 cells in mice of both strains. DNA synthesis was inhibited more in the target cells during their confrontation with splenocytes of mice receiving B 58 (Fig. 1b). Activity of cytostatic effectors in CBA mice was maximal 1 day after injection of 100 μg of compound B 58 and during the 2 days after injection of B 58 in a dose of 10 μg. This was followed by a decrease of activity to its initial level. In A/Sn mice a peak of activity of the cytostatic effectors was observed 1 day after injection of B 58 in both doses, whereas after 2 days activity fell, and it rose again during the next 2 days.

With the aid of compound B 58 it is thus possible to discriminate between the function of NKC and of cytostatic spleen cells. The increase in activity of the cytostatic cells in A/Sn mice, with the absence of any effect of B 58 on the NKC system, may indicate high sensitivity of cytostatic cells to interferon (low threshold of sensitivity) or realization of a mechanism that is unconnected with interferon involvement. The complex character of the kinetics of the potentiation of cytostatic cell activity in A/Sn mice may be due to heterogeneity of the population of this class of cells in mice of this strain or to the presence of a regulatory mechanism that so far has bot been studied.

During the investigation of induction of interferon formation in mice by injection of B 58, it was shown that the peak of interferon accumulation in the serum (320 U/ml) occurs after 6-24 h. Even on the 7th day, however, trace amounts of interferon were found in the serum (Fig. 2). The interferon obtained was resistant neither to acid nor to heat, which characterizes it as type II interferon or γ-interferon. Correlation was found between the increase in NKC activity and the rise of the serum interferon level under the influence of B 58. As a powerful interferon inducer, B 58 evidently activates NKC by an interferon-dependent mechanism [6, 12].

The next step was accordingly to compare B 58 with another NKC activator, polyI:C, whose action is realized through interferon production. The direct action of α-interferon on NKC activity also was investigated. (CBA × C57BL/6)F_1 mice received an intraperitoneal injection of compound B 58, polyI:C (both in a dose of 100 μg/mouse), or α-interferon ($2.5 \cdot 10^3$ U/mouse). It can be concluded from the data given in Fig. 3 that compound B 58 is similar in the effectiveness of its action to polyI:C and α-interferon. Potentiation of NKC activity by compound B 58 is evidently realized through interferon production.

Compound B 58 also was tested as a possible inducer of interleukin 2. Compound B 58 (10 and 20 μg/ml) induced interleukin 2 production much less effectively (indices of stimulation 6.75 and 7.6, respectively) than con A (5 μg/ml; stimulation index 321), the classical inducer of this lymphokine.

The results are in agreement with the view that interferon and interleukin 2 are involved in the regulation and maintenance of NKC activity [7-9].

We consider that the increase in activity of the cellular mechanisms of natural antitumor resistance arising under the influence of compound B 58 is a very important fact. It provides a basis for the use of B 58 in the treatment of neoplasms.

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