It can be concluded from these results that hyporeactive cells secrete a factor inhibiting interferon production into the incubation medium. This factor differs from interferon in its antigenic composition. Further investigations will establish its nature and its connection with the intracellular repressor. Besides hyporeactivity factor, interferon itself can evidently control its production by the feedback principle. Two opposite phenomena, namely "priming" and "hyporeactivity," due to interferon, have been described in the literature. The mechanisms whereby interferon regulates its own production are unknown.

LITERATURE CITED

1. F. I. Ershov and A. S. Novokhatskii, Interferon and Its Inducers [in Russian], Moscow (1980).
2. É. B. Tazulakhova and F. I. Ershov, Vopr. Virusol., No. 6, 703 (1978).
3. É. B. Tazulakhova et al., Antibiotiki, No. 2, 145 (1980).
4. W. E. Stewart II, The Interferon System, Vienna (1979).
5. I. Vilček et al., J. Virol., 10, 614 (1972).

TIME COURSE OF ACTIVITY OF NATURAL MOUSE SPLEEN KILLER CELLS AFTER PARTIAL SPLENECTOMY

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The mouse spleen is capable of reparative regeneration after removal of a large part of the organ. The operation and repair processes in the spleen are accompanied by a drastic fall in the ability of splenocytes to form antibodies, and this is accompanied by changes in the content and functional activity of the T and B lymphocyte populations [3].

In recent years many investigators have directed their attention toward normal natural killer cells (NKC), cells mediating natural cell cytotoxicity. The reasons are not only that NKC are possible effectors against malignantly transformed cells, but also the suggestion that they may play an important role in the maintenance of homeostasis, with their participation in the regulation of normal proliferation and differentiation in renewing systems [4]. The writers showed previously that repair processes in the liver are accompanied by marked changes in NKC activity in the spleen, a reduction of natural cytotoxic activity during the 2 days after the operation, followed by an increase in this activity on the 5th-9th day [1].

The aim of this investigation was to study the dynamics of NKC activity during reparative regeneration of the spleen.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 16-20 g. Under ether anesthesia two-thirds of the spleen was removed from the animals. As a special control, in a separate series of experiments the right submandibular salivary gland, which is incapable of regeneration under these experimental conditions, was completely removed [2]. The animals were decapitated at various times after the operation and cell suspensions prepared from the spleens.

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

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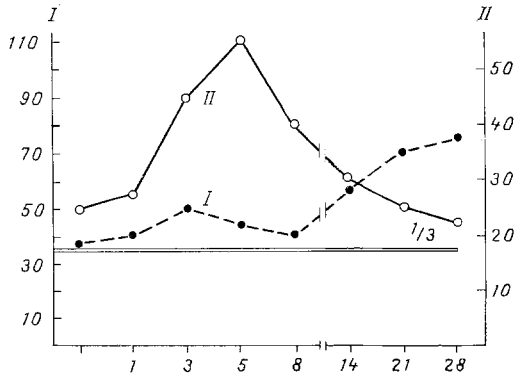

Fig. 1. Time course of number of cells (I) and [^{3}H]thymidine incorporation (II) in mouse spleen regenerating after partial resection. Abscissa, time after operation (in days); ordinate: I) number of cells in spleen ($\cdot 10^6$), II) number of counts per minute for $2 \cdot 10^6$ cells ($\cdot 10^{-3}$). Each point represents mean for 4-6 animals.

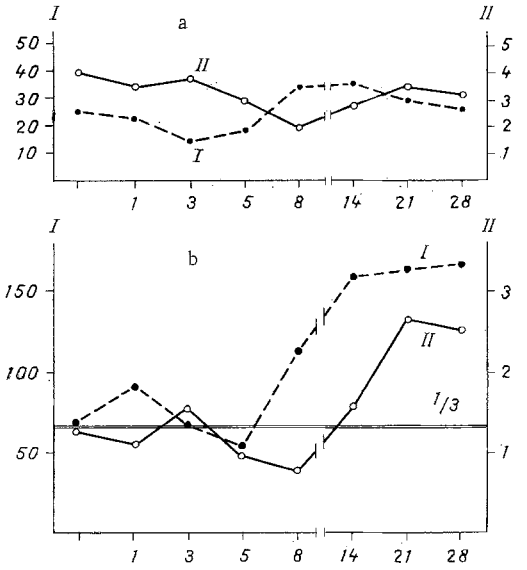

Fig. 2. Dynamics of NKC activity and number of B lymphocytes binding the hapten (TNP) in mouse spleen regenerating after partial resection. Abscissa, time after operation (in days); ordinate: a: I) CI (in %) for ratio EC:TC = 50:1, II) number of TNP-RFC per 10^6 cells ($\cdot 10^{-5}$); b: I) number of lytic units (LU_{20}) per spleen, II) number of TNP-RFC per spleen ($\cdot 10^{-5}$). Here and in Fig. 3 each point represents mean for 6-8 animals.

Natural cytotoxic activity of the spleen cells was determined three times in a test based on liberation of ^{51}Cr from labeled target cells (TC); these were cells of a VAC-1 mouse lymphoma transplanted in vitro. TC, numbering $5 \cdot 10^6$, were incubated with 100 μCi of $Na_2{}^{51}CrO_4$ (specific radioactivity 1 mCi/ml, from Amersham Corporation, England) for 60 min at 37°C. TC numbering $2 \cdot 10^7$ in 0.1 ml of medium, were transferred into wells in round-bottomed plates with an equal volume of effector cells (EC) in the ratios of 1:100, 1:50, and 1:25.

The cells were incubated for 4 h at 37°C. The plates were then centrifuged at 200 g for 3 min. Radioactivity of 0.1 ml of supernatant was measured on a Rack-Gamma gamma-counter. The cytotoxic index (CI) was calculated by the equation (in %):

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

To estimate the lytic potential of the whole organ, cytotoxicity was expressed in lytic units (LU). The number of spleen cells producing lysis of 20% of TC (LU_{20}) was taken as the unit. The control of activity due to NKC was absence of cytotoxicity of the cells against TC resistant to NKC, namely VAC and P-815 cells transplanted in vivo.

To assess changes in subpopulations of specialized B lymphocytes, which could be precursors of antibody-forming cells, the number of lymphocytes carrying receptors against a hapten – the trinitrophenyl (TNP) group – was determined by the rosette formation method with sheep's red blood cells conjugated in TNP [6].

The statistical analysis of the results were carried out by Student's test.

EXPERIMENTAL RESULTS

Results of counting cells remaining in the stump of the spleen after resection of two-thirds of the organ and of determination of [^{3}H]thymidine incorporation by these cells at different time intervals after the operation are given in Fig. 1. In the resected organ there was an increase in incorporation of the labeled DNA precursor, which was most marked between the 3rd and 8th days. Restoration of the number of cells in the regenerating spleen was slow, and by the 28th day it had reached only two-thirds of their number in the intact organ.

The level of NKC activity in the resected spleen fell significantly ($P < 0.05$) by the 3rd day (Fig. 2a). However, 8 days after the operation NKC activity was higher than the level of cytotoxic activity of NKC from the intact spleen ($P < 0.05$). When the cytotoxic activity of NKC was expressed in lytic units and the lytic potential of the whole organ estimated, it could be seen to be considerably greater than the lytic activity of one-third of the normal spleen, but below the potential of the whole intact spleen (Fig. 2b). The time course of NKC activity differed from the change in content of the other lymphocyte subpopulation (specialized B lymphocytes binding the hapten) in the resected organ. In the early period after the operation the fraction of TNP-RFC in the spleen cells showed little change. It was reduced by half by the 8th day, but then gradually increased, to reach 80% of the control level by the 21st day. However, the number of TNP-RFC in the whole regenerating spleen barely reached 60% of their number in the intact spleen.

Unilateral removal of a paired nonlymphoid organ, the submandibular salivary gland, also led to a change in the number of cells, activity of NKC, and the number of TNP-RFC in the spleen of the animals undergoing the operation (Fig. 3). By the 3rd day after the operation a decrease in NKC activity was observed. However, starting with the 5th day, its level rose to reach a maximum on the 8th day. By that time the lytic activity of the spleen of the animal undergoing the operation was significantly higher than that of the intact animal. The number of TNP-RFC in the spleen of the sialadenectomized mice also was reduced after the operation. The number of these cells was restored after 28 days, much more slowly than NKC activity.

The results are evidence of a quantiatively different response to trauma and a different time course of regeneration of the cells of the two different lymphocyte subpopulations, performing different functions. Activity of NKC, to which an important role is ascribed in the antitumor resistance of the organism [5] and possible participation in the regulation of proliferation and differentiation of normal cells [4], fell sharply during the first 3 days. By the 8th day the level of NKC activity was higher than its level in the intact spleen, and subsequently it remained comparable with it. Consequently, at least 8 days is required for NKC activity to be restored in the resected spleen. However, since complete restoration of the number of cells in the organ did not take place, the regenerated spleen had lower lytic potential. The fraction of B lymphocytes carrying specific receptors for the hapten, i.e., the fraction of precursor cells capable of synthesizing specific antibodies against that hapten after stimulation with antigen, showed little change in the early stages after the operation. The greater decrease in the fraction of TNP-RFC occurred later, toward the 8th day, and their number was not completely restored in the course of 28 days. The depressed humoral response of cells of the regenerating spleen [3] may evidently depend not only on a decrease in the fraction of T-lymphocytes and a change in the ratio of their subpopulations, but also on a decrease in the number of precursors of antibody-forming cells.

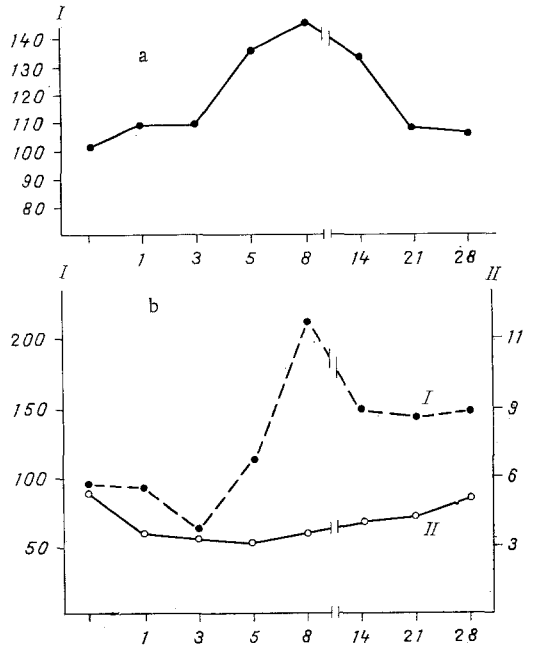

Fig. 3. Dynamics of number of cells, activity of NKC, and number of TNP-RFC in mouse spleen after unilateral removal of submandibular gland. Abscissa, time after operation (in days); ordinate: a) number of cells in spleen ($\cdot 10^{-6}$); b: I) number of lytic units (LU_{20}) per spleen; II) number of TNP-RFC per spleen ($\cdot 10^{-5}$).

The dynamics of NKC activity in the regenerating spleen differed considerably from changes in its activity in the mouse spleen after removal of a salivary gland, an organ not directly connected functionally with the spleen. In this case, after an initial decrease there was a rapid and more marked increase in NKC activity. The total lytic activity of the spleen in these animals after 8 days was much greater than the lytic potential of the spleen in intact mice. Similar changes, but quantitatively more marked still, were observed during reparative regeneration of the liver [1]. The absence of restoration of the total lytic potential of the regenerating spleen may be linked with removal of a large part of the stroma of the organ, leading to a deficiency of cells which constitute the microenvironment, and the absence of free "planting sites."

During regeneration in the spleen changes are thus observed in the level of activity of NKC, manifested as a decrease in the early period after partial resection of the organ and a relatively rapid rise in the course of 8 days. However, the total lytic potential of the regenerating spleen is not restored in the course of 28 days.

LITERATURE CITED

1. A. P. Avtsyn, A. G. Babaeva, L. V. Van'ko, et al., Dokl. Akad. Nauk SSSR, 271, No. 6, 1514 (1983).
2. A. G. Babaeva and E. A. Shubnikova, Structure, Function, and Adaptive Growth of the Salivary Glands [in Russian], Moscow (1979).
3. G. V. Kharlova, Regeneration of Lymphoid Organs in Mammals [in Russian], Moscow (1975).
4. G. Cudkowicz and P. S. Hochman, Immunol. Rev., 44, 13 (1979).
5. R. B. Herberman and H. T. Holden, J. Natl. Cancer Inst., 62, 441 (1979).
6. M. B. Rittenberg and K. L. Pratt, Proc. Soc. Exp. Biol. (N.Y.), 132, 575 (1969).