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INCREASED IMMUNOLOGIC REACTIVITY OF MTV-INFECTED C3H MICE AGAINST HEPATOMA 22a CELLS COMPARED WITH MTV-FREE (C3Hf) MICE

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KEY WORDS: MTV-infected (C3H/He) and MTV-free (C3Hf) mice; antitumor immunity; natural killers.

A virus of mammary gland carcinoma (MTV) is known to persist permanently in mice of certain strains and is the cause of the high frequency of mammary gland tumors in these animals [1, 15, 9, 13]. It has been shown that MTV-infected mice can easily be freed from the virus if the newborn mice are fed with milk from MTV-free mice of low-cancer lines [8]. As a rule, mammary gland tumor cells in MTV-infected animals grow more rapidly than in MTV-free animals. This is because of the stronger immune response of the MTV-free mice to antigens (MTV) present on such tumor cells [3, 10, 14, 16]. In the course of experiments with named sublines of the C3H strain of mice the present writers found that cells of some tumors of different tissue origin, including hepatoma 22a, if inoculated into mice of these sublines, behave in the directly opposite manner, i.e., they grow much less readily on MTV-infected than MTV-free animals.

The object of this investigation was to study the phenomenon of slower growth of hepatoma 22a cells in MTV-infected mice.

EXPERIMENTAL METHOD

Male mice aged 2-4 months belonging to MTV-infected (C3H/He) and MTV-free (C3Hf) sublines of the C3H strain were used. The C3Hf mice were obtained in the writers' laboratory by the standard method [8], fully described previously [2]. The following cell lines of the C3H phenotype, obtained by passage *in vitro* in the writers' laboratory, were used: 1) nonmalignat L cells (fibroblasts transplanted *in vitro*); 2) the malignant subline LS of these cells; 3) two cell lines of spontaneous mammary gland tumors — MMT1 and MT5; 4) cells of hepatoma 22a.

The animals were immunized by subcutaneous injection of $1 \cdot 10^6$ hepatoma cells into each limb. Lymph node cells were obtained 9 days, spleen cells and serum 14 days after immunization. The cytotoxic test with lymphocytes was carried out by the method described previously [4], by incubating effector and target cells for 48 h in an atmosphere containing 5% CO_2 at 37°C. The concentration of lymph node cells in the vessels was $2 \cdot 10^6$, and of splenocytes $1 \cdot 10^6$ cells/ml. In some variants of the experiments the target cells were irradiated to prevent them from dividing [4]. The cytotoxic test with freshly obtained rabbit complement (dilution 1:10-1:15) was carried out by the method in [6]. Cytotoxic activity of the lymphocytes and sera was determined by counting the number of living cells left on the slide after the reaction. In each variant of the experiment data at least 12 experimental and six con-

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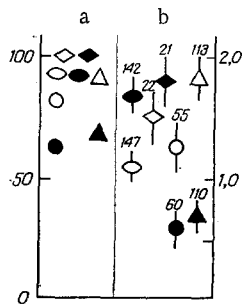

Fig. 1

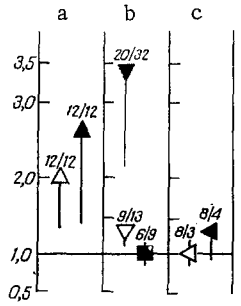

Fig. 2

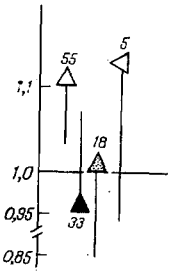

Fig. 3

Fig. 1. Take rate (a) and mean weight of tumors of different types (b) in C3Hf (empty symbols) and C3H/He (filled symbols) mice. Tumors: MMT1 (ellipse); MT5 (lozenge); LS (circle); hepatoma 22a (triangle). Ordinate: on left, take rate of tumors (in %); on right, weight of tumor (in g). Numbers above symbols give number of animals in experiment. Vertical lines denote standard error. Mice killed 30 days after subcutaneous inoculation with $1 \cdot 10^6$ LS and hepatoma 22a cells or $0.5 \cdot 10^6$ MT5 and MMT1 cells in the spinal region.

Fig. 2. Cytotoxic reaction of lymph node lymphocytes (a), splenocytes (b), and sera with complement (c) of C3Hf (empty symbols) and C3H/He (filled symbols) mice after inoculation with hepatoma 22a cells. Triangles — target cells of hepatoma 22a; square — L target cells; a and c — living target cells, b — cells irradiated in a dose of 4000 R. Ordinate, ratio of cytotoxic activity in animals of control group to that in experimental animals. Numbers above symbols denote number of control (left) and experimental (right) animals or samples of sera studied. Vertical lines show confidence intervals at $P \leq 0.05$.

Fig. 3. Comparison of natural cytotoxicity of lymphocytes and sera of C3Hf and C3H/He mice against hepatoma 22a cells. Ordinate — ratio of cytotoxic activity of C3Hf mice to that of C3H/He mice. Effectors as follows: 55) lymph node cells, 33) unfractionated suspension of spleen cells; 18) spleen cells without macrophages (macrophages separated by sedimentation twice on slide; 5) antiserum with complement. Numbers above symbols give number of pairs of animals tested. Vertical lines show confidence intervals at $P \leq 0.05$.

trol animals and at least three samples of sera were compared. The results of identical experiments were pooled and subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

As Fig. 1 shows, mammary gland tumors grew at the same rate (MT5; $P > 0.1$) or significantly faster (MMT ; $P < 0.1$) in C3H/He mice than in C3Hf mice. On the other hand LS (sarcoma) cells and hepatoma 22a cells took and grew in the C3H/He mice much less readily than in C3Hf mice ($P < 0.001$). The difference was particularly clear in hepatoma 22a cells (Fig. 1). Since the cells of this tumor grew much better in animals irradiated in a dose of 600 R (the mean weight of the tumors in the control and irradiated C3H/He mice was 0.15 ± 0.04 and 1.33 ± 0.19 g, respectively), it is logical to suggest that they induce immunity in the recipients. Accordingly, the degree of this immunity was compared in mice of the two sublines.

Data showing the degree of cytotoxicity of the lymphocytes and sera of C3Hf and C3H/He mice after inoculation with hepatoma 22a cells show (Fig. 2) that mice of both sublines developed a marked cellular immune response 9 days after injection of the cells; in the C3H/He mice this response was stronger than in C3Hf mice. The difference was particularly clear in the response of the splenocytes. The immunologic response to hepatoma cells was specific, for the splenocytes did not exhibit any cytotoxic reaction on other target cells (Fig. 2).

It thus follows from the results described above that C3H mice infected with MTV virus develop a stronger immune response against tumor cells not carrying antigens of this virus. In the last six years a number of workers have isolated and studied a new class of lymphocytes, which can give a cytotoxic reaction against tumor cells without preliminary immunization [7, 12, 17]. It has been shown that infection of animals with certain viruses may lead to an increase in this natural killer activity [17]. The presence of natural cytotoxic anti-

bodies has been demonstrated in mice in other investigations [5]. If such a natural cellular and (or) humoral cytotoxicity can be demonstrated in MTV-infected mice, it could itself be the cause of the slower growth of certain tumors and, in addition, it could contribute to the development of the stronger specific immune response to cells of such tumors, as has previously been suggested during an examination of other systems [11].

The cytotoxic activity of lymph node and spleen cells and also of the sera of intact C3Hf and C3H/He mice against hepatoma 22a cells was compared. The results show (Fig. 3) that lymph node cells of mice infected with MTV virus in fact had a significantly stronger cytotoxic effect on hepatoma 22a cells than lymphocytes of C3Hf mice. The splenocytes and sera of these animals did not differ statistically significantly in their activity.

The lower take rate and the lower rate of growth of cells of MTV-free tumors in MTV-infected animals may thus be the result of the enhanced immune response of these animals to tumor cells free from MTV antigens. In turn, it is logical to suggest that the high specific immunologic reactivity of the MTV-infected mice is the result of the raised level of natural killer activity of these animals against the above-mentioned cells.

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