ABSENCE OF GENERALIZED IMMUNOSUPPRESSION IN C57B1/6 MICE DURING PROGRESSIVE GROWTH OF SYNGENEIC T-LYMPHOMA EL-4 AND OF LEWIS 3LL LUNG CARCINOMA

B. B. Fuks, I. M. Malaitsev,
and I. M. Bogdanova

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Inability of the immune system to reject autologous or syngeneic tumors may be due to the absence of specific immune recognition or depression of the effector systems of immunity [11, 13]. Besides specific suppression, generalized depression of immune functions as a result of tumor growth also has been described [8]. Nonspecific lowering of immunity in tumor-bearing animals is manifested mainly in the T lymphocyte population [6, 9]. In particular, it is expressed as lowering of the level of the proliferative response of lymphocytes to T-cell mitogens and alloantigens [6]. This phenomenon, however, is evidently not universal for all histological types of tumors, and for that reason, in order to work out a strategy of immunotherapy, it is essential to analyze the immune system of the tumor carrier in each individual case. Moreover, testing of the proliferative response of T cells for mitogens is limited from the informational point of view, for it does not answer the question of disturbances within individual T-cell populations. Nevertheless, participation of cytotoxic T lymphocytes (CTL), effectors of delayed hypersensitivity, and T amplifier cells in antitumor immunity has been demonstrated in various model systems.

In the investigation described below the proliferative response of lymphocytes to mitogens and the generation of allospecific CTL in vivo were investigated in C57BL/6 mice during progressive growth of syngeneic T lymphoma EL-4 and Lewis carcinoma 3LL.

METHODS

Experiments were carried out on 10-12-week old male C57B1/6 mice into whose thigh $2.5 \cdot 10^5$ EL-4 or 3LL cells were injected subcutaneously. The EL-4 tumor was maintained in the ascites form, whereas the 3LL tumor was implanted intramuscularly in a dose of $5 \cdot 10^6$ cells per mouse. Interleuken 2 (IL 2) was obtained and tested by the method in [2, 10]. Mouse spleen cells ($5 \cdot 10^6$ in 1 ml) were incubated with concanavalin A (con A, 5 ug/ml) in medium RPMI-1640 with 5% embryonic calf serum, glutamine (2mM), and 2-mercaptoethanol ($5 \cdot 10^{-5}$M) for 24 h at 37°C in a CO_2 incubator. IL 2 activity was determined in the supernatant culture fluid after sedimentation of the cells by centrifugation at 400 g. IL 2 activity was estimated from the incorporation of ^{3}H-thymidine into 4-day T blast cells. T blast cells, in a volume of 100 µl (10^6 in 1 ml) were added to the wells of flat-bottomed culture panels, to the test samples of IL 2, previously diluted with medium (100 µl in each case). ^{3}H-thymidine (0.5 Ci in 50 µl, specific radioactivity 20 Ci/mmole) was added to the wells 4 h before the end of the 18-h period of culture. Radioactivity of the samples was determined on a Packard-Tricarb scintillation β-counter, after sedimentation on filters of the material precipiated by TCA.

The mitogenic response was tested with the aid of con A (1 and 2 µg/ml; from Pharmacia Fine Chemicals, Sweden), phytohemagglutinin (PHA, 30 µg/ml; from Serva, West Germany), pokeweed mitogen (PWM, 10 µg/ml), and *Escherichia coli* (50 µg/ml; from Difco, USA).

Spleen cells ($2.5 \cdot 10^6$ in 1 ml) were cultured with mitogens in a total volume of 200 µl in round-bottomed culture panels for 72 h at 37°C in a CO_2 incubator. ^{3}H-thymidine (1 µCi/50 µl, specific radioactivity 5 Ci/nmole) was added 18 h before the end of culture. The proliferative response was tested by measuring incorporation of ^{3}H-thymidine into the TCA-precipitated material on a scintillation β-counter.

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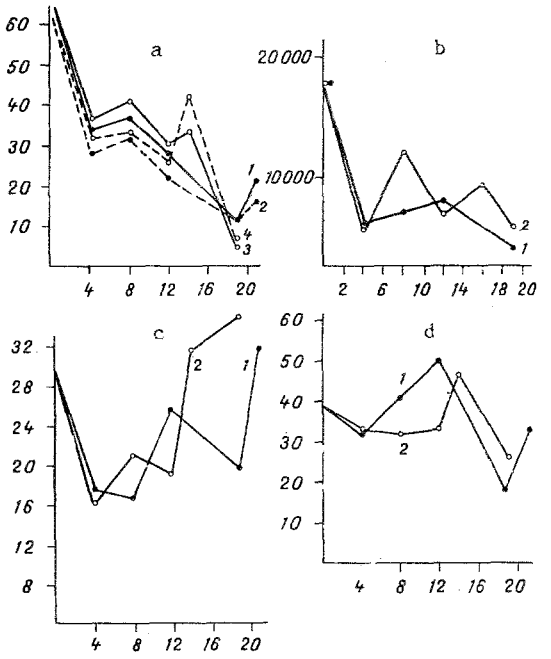

Fig. 1. Mitogenic response of splenocytes of C57B1/6 mice with 3LL and EL-4 tumors. Abscissa, duration of presence of tumor (in days); ordinate, incorporation of ^{3}H-thymidine into cells (in cpm). a) Response to con A; b) response to PHA (30 μg/ml); c) response to PWM (10 νg/ml); d) response to PWM (50 μg/ml). In a: 1) 3LL + 1 μg/ml con A; 2) 3 LL + 2 μg/ml con A; 3) EL-4 + 1 μg/ml con A; 4) EL-4 + 2 μg/ml con A; in b-d: 1) 3LL; 2) EL-4.

In order to generate CTL, $2 \cdot 10^{7}$ allogeneic mastocytome P-815 (H-2^{d}) cells were injected intraperitoneally into tumor-bearing or intact animals (C57B1/6, H-2^{b}) on the 7th day after subcutaneous inoculation of syngeneic EL-4 or 3LL tumor cells. The primary response of CTL was tested 12 days later. Spleen cells of immunized mice were used as the source of CTL in the 4-hourly test of ^{51}Cr release from target cells [1, 4]. The targets were P-815 cells. The ratios of effectors to target cells were 100:1, 50:1, 25:1, 12.5:1, 6.75:1, and 3.375:1.

Activity of CTL was expressed as a percentage of cytolysis or in lytic units [1, 4].

RESULTS

Progressive growth of the EL-4 and 3LL tumors led to weakening of the proliferative responses to T-cell mitogens con A and PHA (Fig. 1, A and B). The tendency toward weakening of themitogenic response was the same in animals with EL-4 and 3LL tumors.

The response to pokeweed mitogen was significantly lower in the early periods of tumor growth and it recovered by the 20th day of testing (Fig. 1c). Recovery of the response was exhibited more completely in mice with an EL-4 tumor. Since PWM induces proliferation of both T and B cells, this suggested that the complex time course of the response to this mitogen may be connected with relative integrity of the proliferative function of the B lymphocytes. However, the results of testing of the response to the B-cell mitogen (PWM) did not confirm this suggestion. The response to PWM was not disturbed in the early stages of tumor growth, but it was considerably weakened on the 19th day (Fig. 1d), i.e., at a time when the response to pokeweed mitogen was restored. Cooperative interactions between T and B lymphocytes in the response to PWM evidently lie at the basis of these differences.

The time course of IL 2 production in mice with tumors coincided with that of the proliferative response to con A (Fig. 2).

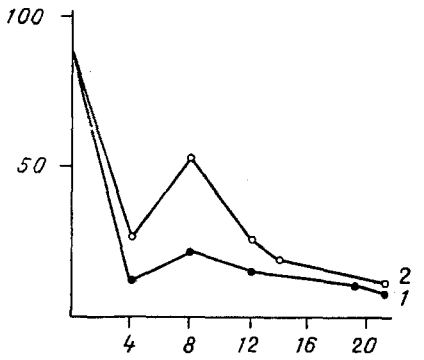

Fig. 2. Con A-induced IL 2 production in mice with tumors. Abscissa, duration of presence of tumor (in days); ordinate, incorporation of ^{3}H-thymidine into indicator cells – 96-h con A-blast cells (in cpm). 1) 3LL; 2) EL-4.

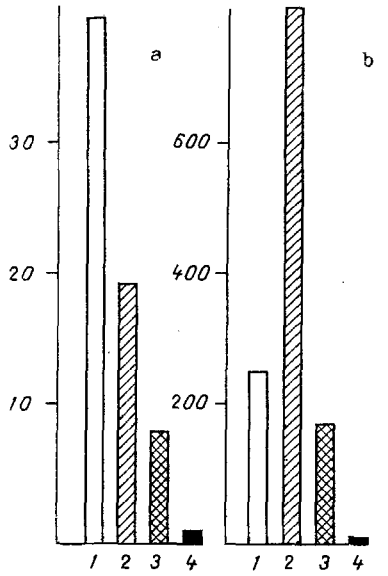

Fig. 3. Splenic CTL response of mice with tumors to allogeneic tumor (mastocytoma P-815). a) Cytolysis of P-815 target cells by lymphocytes of immune mice (ratio effectors/targets = 50:1); 1) immune mice without syngeneic tumor; 2) immune mice with EL-4 tumor; 3) immune mice with 3LL tumor; 4) intact mice without syngeneic tumor. b) Total lytic activity of CTL per spleen. Ordinate, number of lytic units per spleen.

The question arose whether the results could be interpreted as generalized suppression of the T-cell response in animals with tumors. To test this hypothesis the primary response of CPL to an allogeneic graft was studied. Allogeneic P-815 tumor cells ($2 \cdot 10^7$) were injected intraperitoneally into mice 6 days after subcutaneous inoculation with the syngeneic tumor, and CTL activity was estimated 12 days later in the population of spleen cells.

CTL activity was depressed in mice with syngeneic tumors, and more so, moreover, in animals with lymphoma EL-4. However, if allo-CTL activity was expressed per spleen, it was higher in mice with carcinoma 3LL than in animals of the control group, whereas in mice with the EL-4 tumor it was somewhat lower than in the control (Fig. 3).

Hence it can be concluded that the level of IL 2 production in mice with tumors EL-4 and 3LL was high enough to generate an effective CTL response and that the absence of CTL response to a syngeneic tumor cannot be explained by a nonspecific suppressor effect of the tumor carrier state on the immune system. Since EL-4 and 3LL cells carry tumor-specific transplantation antigens [3, 12], the absence of response of CTL to them may be connected with specific immunosuppression. Abnormal expression of products of the H-2 complex on 3LL cells (absence of $H\text{-}2K^b$ glycoprotein) on 3LL cells was demonstrated previously [5, 7]. In the opinion of the authors cited, absence of the restricting elements ($H\text{-}2K^b$) prevents recognition of the tumor-associated antigen, whereas its recognition in the $H\text{-}2D^b$ context leads to generation of specific suppressors.

On the basis of the results of the present investigation showing lowering of the level of the mitogenic response it can be postulated that in the tumor carrier state nonspecific suppression of immunity is manifested also, but a normal response of PTL can develop against its background.

LITERATURE CITED

1. I. M. Bogdanova, G. T. Sukhikh, and V. V. Malaitsev, Immunologiya, No. 1, 21 (1982).
2. G. T. Sukhikh, V. V. Malaitsev, and I. M. Bogdanova, Dokl. Akad. Nauk SSSR, 278, No. 3, 762 (1984).
3. E. Benjamini et al., J. Immunol., 118, 685 (1966).
4. J. C. Cerottini and K. T. Brunner, in: *In vitro* Methods in Cell Mediated Immunity, New York (1971), p. 369.
5. L. Eisenbach et al., Int. J. Cancer, 34, 567 (1984).
6. K. D. Elgert and W. L. Farrar, J. Immunol., 120, 1345 (1978).
7. N. Isakov et al., J. Nat. Cancer Inst., 66, 919 (1981).
8. I. Kamo and H. Friedman, Adv. Cancer Res., 25, 271 (1977).
9. D. G. Kilburn et al., Europ. J. Immunol., 4, 784 (1974).
10. K. J. Lafferty et al., Immunol. Rev., 51, 279 (1980).
11. R. J. North and I. Bursuker, J. Exp. Med., 159, 1295 (1984).
12. A. J. Treves et al., Israel J. Med. Sci., 12 369 (1976).
13. R. D. Wortzel et al., Proc. Nat. Acad. Sci. USA, 81, 2186 (1984).