IMMUNODEPRESSANT ACTION OF ANTI-INTERFERON SERUM

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Interferon is considered to participate in the formation and materialization of the immune response, and any antigenic determinant may be an interferonogen. To confirm the role of interferon in the formation of immunologic reactivity experimentally, an attempt was made to block the action of interferon by means of an anti-interferon serum. Injection of the anti-interferon serum into C57BL/6 mice immunized with L cells reduced the cytotoxic action of the lymphocytes and sera of these mice on target cells.

KEY WORDS: interferon; anti-interferon serum; immunodepression; allergy.

The role of interferon in the formation and materialization of the immune response is evidently an essential one, and any antigenic determinant is ipso facto an interferonogen [1-3, 5, 6, 8, 9].

The writers have shown previously that the rejection of skin allografts in mice can be accelerated by means of interferon, and the cytotoxic action of the lymphocytes of these animals on target cells in culture can be intensified [3, 6]. The possibility of lengthening the survival of an allograft by means of anti-interferon serum has also been demonstrated in principle [5].

To confirm the hypothesis of the participation of interferon in the formation of immunologic reactivity experimentally, an attempt was made to block the action of interferon with the aid of anti-interferon serum against the background of antigenic stimulation (immunization of mice with L cells).

EXPERIMENTAL

Experiments were carried out on C57BL/6 female mice weighing 18-20 g. To obtain anti-interferon serum, a goat was immunized with interferon induced in a culture of L cells by Newcastle disease virus. The interferon-containing culture fluid was purified and concentrated. For this purpose the first batches of interferon were centrifuged at 18,000 rpm for 20 min and then concentrated by means of polyethylene glycol (mol. wt. 40,000). The next batches of interferon were concentrated on Amicon filters: the interferon-containing fluid was passed initially through millipore filters, at first with a diameter of 1.2 μ and later with a diameter of 0.45 μ, and finally it was concentrated through UM-2 ultrafilters at 4°C. The titer of the interferon thus obtained was 6000-8000 units/ml.

Before the immunization began blood was taken from the goat in order to prepare the control serum. Every week the animal was given a subcutaneous injection of 10 ml of the concentrated, purified interferon (60,000-80,000 units). The total period of immunization was about two years. The resulting serum was stored at −30°C.

Before use the serum was adsorbed with a suspension of L cells (3×10^6 cells to 1 ml serum) for 1 h at 37°C, then for 24 h at 4°C and with lymphocytes from lymph glands of C57BL/6 mice under analogous conditions. These adsorbed sera were nontoxic to L cells and lymphocytes in the cytotoxic test.

For titrating the anti-interferon serum a series of double dilutions of the control and immune sera from 1 : 2 to 1 : 256 was prepared in medium No. 199 containing 2% inactivated bovine serum. To each dilu-

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tion of the sera 8 units of interferon were added in an equal volume and the mixture was incubated for 1 h at 37°C and 24 h at 4°C. The contents of each tube were then transferred to a monolayer of L cells and the mixture was incubated for 18-20 h at 37°C, after which 100 CPD_{50} of the test virus was added as the top layer. The reaction was read 24 h after the addition of the virus layer. The maximal dilution that still neutralized the protective action of the interferon was taken as the titer of the anti-interferon serum. The titer of the anti-interferon serum used in the subsequent experiments was 1:64. The control serum had no anti-interferon activity.

The cytotoxic test with lymphocytes was carried out in a culture of L cells [11]. On the 7th-10th day after the beginning of immunization lymph glands were taken from the mice of all groups and a suspension of lymphocytes prepared. The lymphocytes were added to a 24-h monolayer of target cells in the ratio of 50:1. After incubation for 4 h the cells were removed from the slide with 0.25% trypsin solution, a mixture of eosin and trypan blue was added to them, and the number of living (unstained) cells in 1 ml suspension counted. Statistical analysis of the results was carried out with the aid of Student's criterion.

The serum of the mice of the experimental and control groups was investigated in the microcytotoxic test [12]. Terasaki's plates were used (capacity of well 10 μl). Next, 1 μl of the immune and control sera was incubated with L cells (1500 cells in the well). After incubation for 30 min at 37°C, 5 μl complement (undiluted guinea pig serum) was added and the incubation continued for a further 1.5 h. The supernatant was then removed from the wells, the supravital stain added, and after exposure for 3 min the excess dye was removed and the reaction read. The reaction was assessed qualitatively by the number of nonviable cells: −) 0-15% of nonviable cells, + or −) 15-20%, +) 21-30%, 2+) 31-50%, 3+) 51-80%, and 4+) over 81% of nonviable cells.

Four groups of C57BL/6 mice were used. Group 1 received a single subcutaneous injection of (7-10) $\times 10^6$ L cells at each of 11 points of the body. Immediately after these injections the animals were given an intraperitoneal injection of 0.3 ml anti-interferon serum; the mice received the same dose of serum daily for 4 days (a total of 5 injections). The mice of group 2 were immunized in the same way with L cells and this was followed by injection of the control serum obtained from the animal before immunization at the same times and in the same volumes; the mice of group 3 were immunized with L cells only by the scheme described above; group 4 consisted of intact animals.

RESULTS

Cytotoxic Action of Lymphocytes on Target Cells. The lymphocytes of the mice of groups 1-4 were investigated by the cytotoxic test in tissue culture. The results were as follows. In the mice receiving anti-interferon serum after immunization (group 1), the cytotoxic action of the lymphocytes on the target cells was depressed compared with the cytotoxic action of the lymphocytes of the immunized mice receiving normal goat serum (group 2): 97,768 ± 6490 and 59,994 ± 3850 living cells in 1 ml, respectively ($P < 0.01$). In the intact animals (group 4) the number of living cells in 1 ml was 143,419 ± 9900, and in the mice of group 3 the number was 38,885 ± 4730.

Cytotoxic Action of the Sera. The sera of the mice of the experimental and control groups was investigated in the microcytotoxic test (Fig. 1). It will be seen in Fig. 1 that the sera of the mice immunized with L cells, and also the sera of animals immunized with L cells and also receiving an injection of the control serum, exhibited cytotoxic activity against L cells in a dilution of 1:40. Meanwhile, the serum of mice immunized with L cells and receiving injections of anti-interferon serum had no cytotoxic action on the target cells.

These investigations thus showed that injection of anti-interferon serum against the background of antigenic stimulation produces an immunodepressant effect manifested as both cellular and humoral types. These findings confirm the hypothesis that interferon is an essential component for the formation of immunologic reactivity and that the blocking of its action by anti-interferon serum leads to a definite immunodepressant effect. The possibility cannot be ruled out that lymphocytic interferon induced by an antigenic determinant makes the lymphocyte immunocompetent toward the given antigen, and it persists as long as the immunologic memory persists [2, 6]. The writers consider that interferon also participates in the recognition of the antigen by immune lymphocytes, and it may perhaps be an essential component in the mechanism.

The possibility that interferon, also necessary for the mechanism of contact between the immune lymphocyte and target antigen, is present on the target cell likewise cannot be ruled out.

The state of immunodepression, especially in tumors, in the writers' opinion is connected with inhibi-

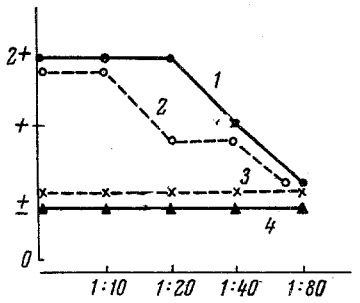

Fig. 1. Cytotoxic activity of sera of C57BL/6 mice against L cells: 1) serum of mice immunized with L cells; 2) serum of mice immunized with L cells and receiving injections of normal goat serum; 3) serum of mice immunized with L cells and receiving injections of anti-interferon goat serum; 4) serum of intact mice. Abscissa, dilutions of sera; ordinate, cytotoxic activity of sera (in conventional units).

tion of interferon formation or with the presence of a repressor of its biological activity. In the latter case the repressor plays a role in blocking the action of the immune lymphocyte on the tumor cell. The writers also consider that excessive interferon formation, not corresponding to the inducing determinant, may cause the development of an autoimmune (allergic) disease. Under these circumstances the excess of interferon may find its way into the blood stream, on to the immune lymphocyte, or on the target cell. As a result, the immune lymphocyte increases the cytotoxicity and the target cell increases its own sensitivity to the action of the immune lymphocyte or immunoglobulin. It likewise cannot be ruled out that under the influence of interferon an excessive number of lymphocytes become implicated in the immune process and the immunologic tolerance to certain autoantigens is reduced. The authors have found interferon in the sera of patients with autoimmune (allergic) diseases.

The development of anaphylactic shock, in the writers' opinion, is connected with increased liberation of interferon by immune lymphocytes and with a larger number of target cells of various nonlymphoid organs in response to a second injection of the original antigen. The increased liberation of interferon by isolated cells in response to repeated local injection or introduction of antigen leads to local allergy (the Arthüs phenomenon, etc.). The immune lymphocyte has been shown to secrete an increased quantity of interferon in response to repeated injection of the specific antigen [8, 9], and treatment of an immune lymphocyte with interferon increases its cytotoxicity.

It is concluded that there are good grounds for using anti-interferon serum (immunoglobulin) in autoimmune (allergic) diseases and also in states in which immunodepression has to be induced [7]. By prolonged immunization of a donkey with human leukocytic interferon an active anti-interferon serum was obtained [4], and immunoglobulins of high interferon-disintegrating activity were isolated from it. Preliminary tests have demonstrated the therapeutic activity of anti-interferon immunoglobulins in autoimmune (allergic) diseases in man.

LITERATURE CITED

1. G. Ya. Swett-Moldavskii and I. Yu. Chernyakhovskaya, Dokl. Akad. Nauk SSSR, 175, 224 (1967).
2. S. V. Skurkovich and I. M. Aleksandrovskaya, Probl. Gematol., No. 3, 34 (1973).
3. S. V. Shurkovich, É. G. Klinova, I. M. Aleksandrovskaya, et al., Byull. Éksperim. Biol. i Med., No. 4, 66 (1973).
4. S. V. Skurkovich, E. I. Eremkina, and É. G. Klinova, Vopr. Virusol., No. 2, 241 (1974).
5. S. V. Skurkovich, É. G. Klinova, I. M. Aleksandrovskaya, et al., in: Proceedings of the 13th Congress of the International Society of Blood Transfusion, Washington (1972), p. 39.
6. S. V. Skurkovich, É. G. Klinova, I. M. Aleksandrovskaya, et al., Immunology, 25, 317 (1973).
7. S. V. Skurkovich, É. G. Klinova, E. I. Eremkina, et al., Nature, 247, 551 (1974).
8. J. A. Green, S. R. Cooperband, and S. Kibrick, Science, 164, 1415 (1969).
9. J. A. Green, S. R. Cooperband, and F. K. Laurence, Ann. New York Acad. Sci., 173, 736 (1970).
10. P. Lindahl, P. Leary, and I. Gresser, Proc. Nat. Acad. Sci. (Washington), 69, 721 (1972).
11. W. Rosenau and H. D. Moon, J. Nat. Cancer Inst., 27, 471 (1961).
12. P. I. Terasaki and J. D. McClelland, Nature, 204, 998 (1964).