THYMECTOMY AND SPLENECTOMY IN ADULT MICE DO NOT AFFECT SUPPRESSOR ACTIVITY OF T LYMPHOCYTES SPECIFIC FOR ANTIGENS OF THE H-2 COMPLEX

V. V. Kronin and N. G. Anosova

UDC 612.112.94.017.1.06:[612.438+612.44].089.64

KEY WORDS: thymectomy, splenectomy, T lymphocytes, antigen of the H-2 complex.

Intravenous immunization with allogenic splenocytes can lead to the induction of tolerance in the recipient to the donor's alloantigens [9]. One cause of the formation of tolerance during intravenous immunization of mice with a large dose of irradiated allogeneic splenocytes is induction of T lymphocytes, possessing specific suppressor activity (SSA) relative to the donor's alloantigens [2, 6]. It has been shown that in this system SSA possess only T lymphocytes [7]. SSA, induced in this particular system were tested both in vivo, with respect to inhibition of the delayed-type hypersensitivity reaction (DTH) [6] and in vitro, relative to suppression of the proliferative response in mixed lymphocyte culture (MLC) [2]. It is probable that these types of immune response may be suppressed by the same effector T-cell population, carrying Thy-1 and Lyt2 markers [1, 6]. However, when different testing systems were used, differences were found. For instance, on testing in vivo SSA of lymphocytes was discovered both in the spleen and in the lymph nodes (LN) of immune mice [6], whereas on testing in vitro they were found in the spleen but not in LN [2]. Moreover, thymectomy (TE) in adult mice completely abolished subsequent induction of SSA, as shown by the results of testing for it in MLC [4], but it had no effect on the level of suppression of the DTH reaction [6]. Accordingly, precursors of the suppressing effectors were placed in the category of short- and long-living T lymphocytes, respectively.

In the present investigation the effect of TE and splenectomy (SE) in adult mice on the level of suppressor activity of T lymphocytes specific for antigens of the H-2 complex was studied.

METHODS

Mice of inbred lines BALB/c (H-d^d), C57Bl /6 (B6) (H-2^b), B10.M (H-2^f), and SIL (H-2^s) were obtained from the nursery of the All-Union Oncologic Scientific Center, Russian Academy of Medical Sciences. SSA of the T lymphocytes was induced in BALB/c mice by a single intravenous injection of $9 \cdot 10^7$ B6 mouse spleen cells, irradiated in a dose of 1500 rad ($^{137}C_s$, 740 rad/min, Stebel' apparatus). SSA was determined on the 4th day after immunization [2]. It must be recalled that under these conditions, besides suppressor activity, in certain cases specific killer activity may be induced. The term SSA is retained because of the use of the corresponding function test in this investigation, and also in connection with the established terminology [2, 6]. The reaction in MLC [3] was carried out by incubating a mixture of $3 \cdot 10^5$ BALB/c mouse LN cells (reacting lymphocytes) with 10^6 spleen cells (stimulators), in round-bottomed wells of 96-well plates (Linbro), of mice of the following lines: B6 — donor, B10.M, SIL — xenogenic, and BALB/c — syngeneic.

Laboratory of Mechanisms of Regulation of Immunity, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences, Moscow. (Presented by Academician N. N. Trapeznikov, Academy of Medical Sciences.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 4, pp. 392-395, April, 1992. Original article submitted September 19, 1991.

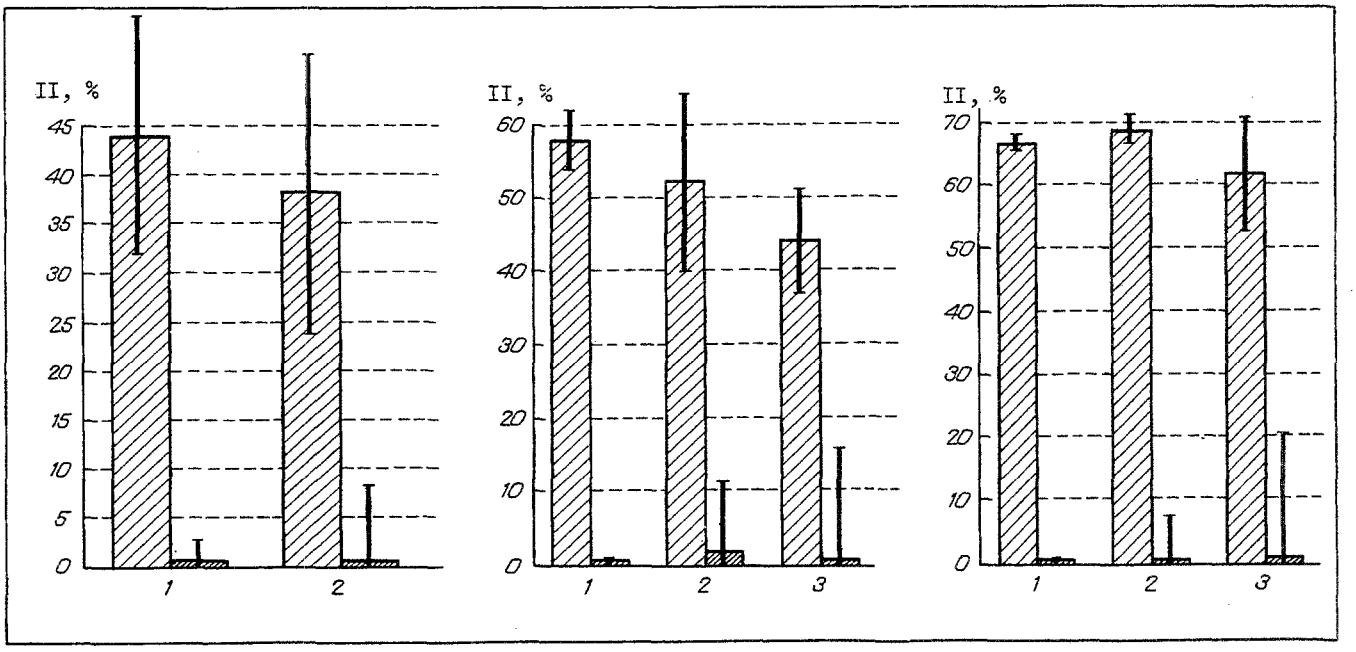

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Comparison of values of SSA induced by intravenous immunization in lymphocyte population from recipient's spleen and LN. SSA of lymphocytes from spleen (1) and LN (2) of immunized (intact in the control) BALB/c mice was determined by the use of stimulators of donor's B6 (pale columns) and xenogeneic B10.M and SIL (dark columns) lines. Ordinate, II (in %). Average values and standard errors based on results of three experiments are shown.

Fig. 2. Effect of SE on intravenous induction of SSA. SSA of LN lymphocytes of immune (intact in control) BALB/c mice was determined in MLC with immunization of intact (1), MO (2), and SE (3) recipients. SE and MO performed 4 weeks before immunization. SSA was determined in MLC by the use of donor's B6 stimulators (pale columns) or xenogeneic B10.M (dark columns) lines. Ordinate, II (in %). Mean values and standard errors based on results of four experiments.

Fig. 3. Effect of TE on intravenous induction of SSA. SSA of splenic lymphocytes of immune (intact in the control) BALB/c mice was determined in MLC using stimulators of donor's B6 (pale columns) or xenogeneic B10.M (dark columns) lines. Value of SSA determined on immunization of intact (1), MO (2), and TE (3) recipients. Ordinate, II (in %). Mean values and standard errors based on results of three experiments shown.

Associates), $5 \cdot 10^{-5}$ M 2-mercaptoethanol (Calbiochem), and 100 U/ml of gentamicin (Farmakhim). ^{3}H-Thymidine was added to the cultures in a dose of 1 μCi/well 16 h before the end of culture. To determine SSA in MLC, $(1\text{-}2) \cdot 10^5$ spleen cells or LN cells of immune (intact in the control) BALB/c mice, treated with mitomycin C (25 μg/ml, 30 min) (Sigma), were added. SSA was estimated as the inhibition index (II), calculated by the equation

$$II = (1 - [CPM_{al}^{e} - CPM_{syn}^{e}]:[CPM_{al}^{c} - CPM_{syn}^{c}]) \cdot 100\%,$$

where CPM_{al}^{e} and CPM_{syn}^{e} denote incorporation of ^{3}H-thymidine (in cpm) in MLC with the addition of immune lymphocytes in response to allogeneic and syngeneic stimulators, respectively; CPM_{al}^{c} and CPM_{syn}^{c} are the same parameters for addition of intact lymphocytes as the control. TE, Se, and the mock operation (MO) in the control [11] were performed on the BALB/c mice at the age of 6-8 weeks. Immunization was carried out 4 weeks after SE and 6 weeks after TE. The time of 6 weeks after TE is considered to be sufficient for the removal of short-living T lymphocytes [14].

RESULTS

In this investigation the problem studied was whether LN of intravenously immunized mice, like the spleen and thymus [2], is a region containing T lymphocytes which possess SSA in MLC. Accordingly, as suppressor cells in MLC we used population of spleen cells and LN cells in parallel series.

As Fig. 1 shows, the population of immune lymphocytes removed from the recipient's LN on the 4th day after intravenous immunization possesses considerable SSA, comparable with activity of a splenocyte population from the same mice.

Since many workers assume a special type of activation of T lymphocytes without involvement of antigen-presenting cells on intravenous immunization and attribute to them a key [8], or even critical [15] role of the spleen in the development of suppression, in the present investigation the role of the spleen was studied in the development of SSA in the system used. For this purpose mice undergoing SE and MO were immunized 4 weeks after the operation, and it was shown that SE does not significantly lower the level of SSA induced in the LN lymphocyte population (Fig. 2).

When mice undergoing TE 6 weeks before intravenous immunization were used as recipients, no appreciable decrease in SSA likewise could be found in the population of immune lymphocytes compared with MO in the control (Fig. 3), i.e., removal of the short-living T lymphocytes as a result of TE in adult mice caused virtually no decrease in induction of suppressor activity.

The results thus disagree with views expressed until now on this system [2, 4]. Meanwhile these results are in good agreement with data relating to induction of SSA of T lymphocytes when the DTH reaction is used as the test system [6], suggesting that the same effector cells are involved in suppression both of the proliferative response in MLC and of the DTH reaction.

Absence of SSA in the population of LN lymphocytes of recipients immunized intravenously, observed in [2], may perhaps be attributed to the use of a different pair of mouse strains as donors and recipients, namely C57Bl/10 and B10.D2, and to a consequent change in the kinetics of SSA induction. In particular, whereas in [2] suppressor activity of the splenocytes was not discovered until the 2nd day, for the B6 and BLAB/c lines used in the present study it could be detected the day after intravenous immunization (results not given).

The difference between the results now described and previous findings, indicating that TE in adult mice completely abolishes induction of SSA [4], can be explained by two circumstances. First, by the use of different time intervals between operation and immunization (whereas in the experiments described above immunization was carried out 6 weeks after TE, in [4] the gap was 16 weeks. Second, in [4] TE was carried out on younger recipients (aged 4-5 weeks).

A key problem is whether introduction of suppressor activity of T lymphocytes is linked with activation of a separate population of suppressor precursors or whether it depends entirely on the conditions of activation. Conditions facilitating induction of suppressor activity of T lymphocytes are not yet clear. In some systems the critical role of the recipient's spleen [10, 15] and thymus [5, 13] in the induction of suppression has been revealed, and abolition of the suppressor activity of T lymphocytes as a result of SE [15] or TE [13] has been demonstrated not only before, but actually immediately after immunization of the recipient.

Some types of immune responses are potentiated after TE in adult animals [1]. TE may correlate with reduction of suppressor activity in connection with the possible short life span of suppressor T-effector cells or their precursors, with their dependence on thymic hormones [5], and also with initial induction of T lymphocytes possessing SSA in the thymus, followed by migration into peripheral lymphoid organs [12].

There is evidence, however, that induction of the suppressor activity of T lymphocytes is not sensitive to SE or TE [6]. Experiments illustrated in Figs. 2 and 3 show that SSA against the donor's alloantigens may be induced in mice subjected both to SE 4 weeks before and to TE 6 weeks before intravenous immunization. Thus we did not find that the presence of the thymus and spleen is essential in the induction of suppression of the proliferative response against H-antigens.

The possibility cannot be ruled out that in this case interaction of precursors of effector T cells with the alloantigen takes place on recirculation pathways, after which the activated T lymphocytes migrate into peripheral lymphoid organs and the thymus [2].

The authors are grateful to B. D. Brondz and T. V. Anfalova for useful remarks when the article was being prepared for publication.

LITERATURE CITED

1. B. D. Brondz, *T Lymphocytes and Their Receptors in Immunologic Recognition* [in Russian], Moscow (1987).
2. B. D. Brondz, A. V. Karaulov, and I. F. Abronina, *Tsitologiya,* **22**, No. 5, 583 (1980).
3. B. D. Brondz, E. Ya. Khachikyan, G. I. Drizlikh, et al., *Byull. Éksp. Biol. Med.*, No. 6, 723 (1977).
4. A. P. Suslov, L. S. Eliseeva, B. D. Brondz, et al., *Immunologiya,* No. 4, 74 (1980).
5. G. L. Asherson, M. Zembala, B. Mayhew, et al., *Europ. J. Immunol.*, **6**, 699 (1976).
6. A. T. J. Bianchi, L. M. Hussaarts-Odijk, T. H. Van der Kwast, et al., *Transplantation,* **37**, 490 (1984).
7. B. D. Brondz, I. F. Abronina, M. V. Zaiceva, et al., *Immunol. Rev.*, **80**, 29 (1984).
8. M. I. Greene et al., *Immunol. Rev.*, **50**, 163 (1980).
9. S. Kitagawa et al., *J. Immunol.*, **144**, 4139 (1990).
10. P. H. Lagrange et al., *J. Exp. Med.*, **148**, 235 (1978).
11. B. B. Mishell and S. M. Shiigi, *Selected Methods in Cellular Immunology,* San Francisco (1980).
12. C. L. Reinisch, S. L. Andrew, and S. F. Schlossman, *Proc. Natl. Acad.Sci. USA,* **74**, 2989 (1977).
13. C. L. Reinisch et al., *Cell. Immunol.*, **58**, 165 (1981).
14. E. Simpson and H. Cantor, *Eur. J. Immunol.*, **5**, 337 (1975).
15. M.-S. Sy et al., *J. Immunol.*, **119**, 2095 (1977).