RESISTANCE OF THE SUPPRESSOR FUNCTION OF T CELLS TO AGENTS WITH ANTIPROLIFERATIVE ACTIVITY

V. M. Pisarev, N. N. Smirnova, and L. A. Pevnitskii

UDC 612.112.94:017.1:612.438].014.46:615.276.2

Injection of cyclophosphamide (CP) in a dose of 50-400 mg/kg into mice immunized with sheep's red blood cells (SRBC) does not significantly reduce the ability of the spleen cells of these mice to suppress the primary immune response when transplanted into intact syngeneic recipients. Irradiation of the donors of immune spleen cells (ISC) in a dose of 900 R or treatment of the ISC in vitro with mitomycin C did not affect their suppressor activity. The supernatant (SN) obtained after ultracentrifugation of sonicated ISC inhibited the primary immune response of intact mice. It is concluded that the suppressor effect of ISC is due to a factor produced by the T cells; active proliferation of these cells is not essential for the realization of its action.

KEY WORDS: *suppressor T cells; suppressor factor; cyclophosphamide; mitomycin C; irradiation.*

The properties and mechanisms of the suppressive effect of T cells inhibiting the immune response have been the subjects of intensive study. The suggestion has been made [3, 9] that suppressor T cells play a role in the regulation of various immune reactions (antibody formation, hypersensitivity of delayed type, reactions of transplantation immunity), but the characteristics of these cells have still been inadequately studied and the available data are contradictory.

Data showing that after immunization of mice with sheep's red blood cells (SRBC) cells capable of suppressing the immune response when transplanted into intact syngeneic recipients appear in the spleen were described previously [2]. On the basis of data in the literature and our own observations [2, 18], these cells were classed as T suppressors. It was therefore interesting to study certain properties of the suppressor cells in that system.

In this investigation the effect of several factors, notably cyclophosphamide (CP), mitomycin C, irradiation, and treatment with ultrasound, on the ability of immune spleen cells (ISC) to suppress the primary immune response was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on adult male CBA, C3H/He, and DBA/2 mice (from the Stolbovaya nursery, Academy of Medical Sciences of the USSR). The mice donating ISC were immunized intraperitoneally with $5 \cdot 10^8$ SRBC. After 14 days a suspension of ISC was obtained and, after the cells had been washed twice with medium 199, they were transplanted into syngeneic intact recipients in a dose of between $2 \cdot 10^7$ and $5 \cdot 10^7$ cells. In some experiments the mice donating ISC were injected with CP in various doses or the donors of ISC were irradiated on the Stebel'-3A apparatus in a dose of 900 R 4 h before transplantation of the ISC. In other experiments the isolated ISC were treated in vitro with mitomycin C (Kyowa, Japan) at a rate of 25 µg/ml for ISC in a concentration of $2 \cdot 10^6$/ml for a period of 20 min

Laboratory of Immunogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 9, pp. 327-330, September, 1977. Original article submitted April 15, 1977.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $7.50.

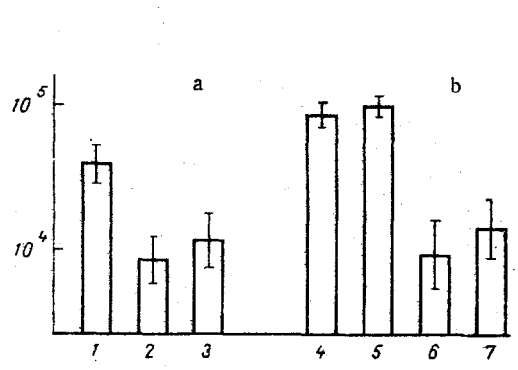

Fig. 1

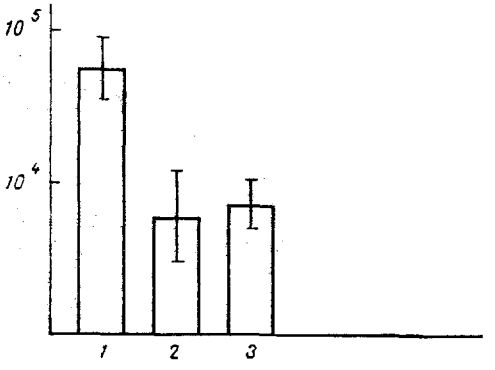

Fig. 2

Fig. 1. Effect of lethal doses of CP (400 mg/kg) and of irradiation (900 R) on suppressor activity of ISC. Here and in Figs. 2 and 3: ordinate, number of 19S AFC in recipients' spleen. a) CP; b) irradiation. 1, 4) SRBC; 2, 6) ISC + SRBC; 3) ISC of donors treated with CP + SRBC; 5) spleen cells of intact irradiated donors + SRBC; 7) ISC of irradiated donors + SRBC. Each recipient injected with $5 \cdot 10^7$ spleen cells. Here and in Figs. 2 and 3 mean geometric values with confidence intervals at $P < 0.05$ given.

Fig. 2. Effect of mitomycin C on suppressor activity of ISC. 1) SRBC; 2) ISC + SRBC; 3) ISC treated with mitomycin C + SRBC. Recipients injected with $2 \cdot 10^7$ ISC.

TABLE 1. Effect of CP on Suppressive Activity of ISC

Donors of spleen cells	Recipients		
	CBA	C3H	
	intact	intact	irradiated
Immune	7 889 (5 395–11 530) $n=15$	5 636 (3 631–8 750) $n=12$	14 450 (6 776–30 830) $n=10$
Immune, treated with CP in a dose of: 50 mg/kg	15 810 (9 462–26 420) $n=8$	9 057 (6 792–12 080) $n=14$	324 (199–527) $n=9$
200 mg/kg	18 620 (12 250–28 310) $n=9$	—	—
Intact, treated with CP (50 mg/kg)	81 610 (63 100–102 600) $n=5$	—	—
Control (only SRBC injected into recipients)	74 470 (56 890–97 500) $n=16$	31 620 (23 710–42 170) $n=14$	—

Legend. Mean geometric numbers of AFC in spleen and confidence intervals at $P < 0.05$ are given; n) number of animals.

at 36°C [4]. In some experiments the recipients were irradiated in a dose of 900 R 4 h before transplantation of the cells into them.

ISC were treated with ultrasound as described previously [16] with certain modifications: The ISC, washed twice by centrifugation, were disintegrated in a concentration of 10^8 cells/ml by ultrasound on the MSE (England) sonicator for 5 min, after which the resulting suspension was centrifuged at 40,000g for 1 h at 4°C. The resulting supernatant (SN) was injected intravenously into syngeneic mice.

The mice were immunized intraperitoneally with $2 \cdot 10^8$ SRBC 15-25 min after injection of ISC or SN. On the 5th day after immunization the number of 19S antibody-forming cells (AFC) was determined by the local hemolysis in agar method [10].

EXPERIMENTAL RESULTS

Results showing the effect of CP on the ability of ISC to suppress the immune response of the intact recipients are given in Table 1. In these experiments CP, in doses of 50 and 200 mg/kg, was injected intraperitoneally into the donors of ISC 3.5-4.5 h before the animals were sacrificed.

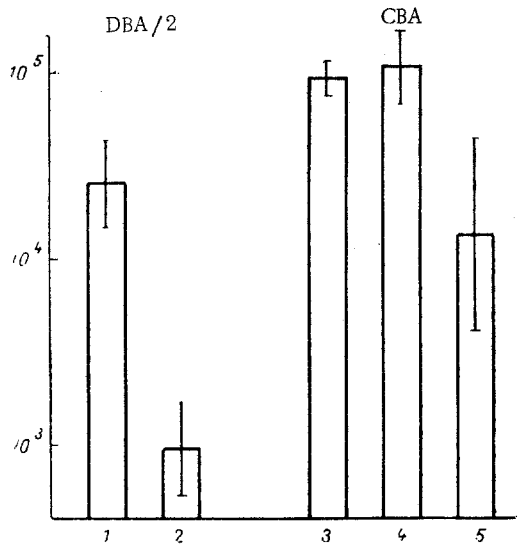

Fig. 3. Action of supernatant (SN) of ISC treated with ultrasound on primary immune response of intact mice. 3) SRBC; 2, 5) SRBC + SN (1 ml); 4) SRBC + SN (0.1 ml).

It will be clear from the data in Table 1 that injection of CP into the donors of ISC did not significantly inhibit the suppressive activity of the cells in the mice of both strains, although the immune response of the ISC themselves, estimated after transplantation into lethally irradiated syngeneic recipients, was virtually completely suppressed. It must be pointed out that even a very high dose of CP (400 mg/kg), if injected into the donors of ISC, did not change the suppressive power of their ISC (Fig. 1a). Injection of CP into the donors in different doses (50-200 mg/kg) 24 h and 4 h before obtaining the suspension of ISC (these data are not shown in Table 1) had equal effects on the suppressive activity of the ISC, which they reduced only partially.

The effect of irradiation in a large dose on the ability of ISC to suppress the immune response of intact recipients is illustrated in Fig. 1b. As this figure shows, irradiation of the donors in a dose of 900 R, 3.5-4 h before transplantation of the cells did not change the suppressive activity of the ISC.

Effective suppression of the immune response also was observed when the ISC were treated with mitomycin C in doses virtually completely suppressing proliferative activity of lymphocytes [4]. As Fig. 2 shows, ISC incubated with mitomycin C suppressed the immune response to the same degree as untreated cells.

Considering the resistance of the suppressor function of ISC to the action of the various agents studied, in the next experiments the ability of the supernatant (SN) obtained after treatment of ISC with ultrasound to suppress the immune response of intact mice was studied. As Fig. 3 shows, injection of SN in a dose of 1 ml, corresponding to 10^8 ISC, led to marked suppression of AFC production in syngeneic recipients. SN in a dose of 0.1 ml was ineffective. The preliminary findings indicate the instability of the suppressive factor contained in SN: Keep it at −20°C for 1 week led to complete loss of its suppressive activity.

The results of this investigation thus demonstrate the high degree of resistance of the suppressor function of ISC to the action of the various agents studied. Similar results were obtained in different experimental systems with respect to mitomycin C [8, 11]. However, the information about the sensitivity of T suppressors to CP and irradiation is contradictory. Most of the work to study the effect of CP on suppressor activity of lymphocytes has been done with reactions of hypersensitivity of delayed type, in which it has been shown that CP may inactivate suppressor cells [12, 14, 15, 19]. A possible explanation is that the suppressors belong to the class of B cells [1, 3], which are known to be more sensitive to CP than T lymphocytes [17].

The results of several investigations support the view that T cells specifically suppressing the immune response are sensitive to irradiation in doses of 650-700 R [6, 7, 19]. In another system, in which nonspecific suppression of the immune response by T cells activated with concanavalin A was observed, the suppressor cells were found to be resistant to irradiation in a dose of 2000 R [13]. There are only isolated items of information on the existence of suppressor cells specifically suppressing the immune response and resistant to irradiation

in a dose of 900 R. Such cells have been found in mice tolerant to human γ-globulin and sensitive to anti-T serum [5].

This ambiguity of the results can evidently be attributed either to differences in the mechanisms of suppression by the T cells or the involvement of different cell subpopulations in this process, depending on the experimental system studied.

The high resistance of the suppressor function of the ISC to the action of chemical and physical agents discovered in this investigation, together with the possibility of suppressing the immune response by SN obtained after destruction and ultracentrifugation of ISC, suggest that proliferation of suppressor cells is not essential in this system for the suppressor effect to take place. These nonproliferating cells contain a factor which suppresses the immune response. The possibility cannot be ruled out that this factor is present on the membrane of the T suppressors, of which there is indirect evidence [16], according to which the suppressive factor obtained after ultracentrifugation of sonicated thymus or spleen cells of mice immunized with a conjugate of dinitrophenol with hemocyanin, has H-2 antigenic characteristics.

The concrete mechanisms of action of the factor of the T suppressors on immunogenesis have received little study. Possibly this factor directly suppresses the activity of the B (or T) cells or acts indirectly, through activating presuppressor T cells of intact mice. This latter hypothesis is supported by certain data on the mechanisms of the suppressive effect of T cells [9, 18].

LITERATURE CITED

1. R. V. Petrov, R. M. Khaitov, and A. A. Batyrbekov, Dokl. Akad. Nauk SSSR, 226, 1446 (1976).
2. V. M. Pisarev and L. A. Pevnitskii, Byull. Eksp. Biol. Med., No. 5, 571 (1977).
3. G. L. Asherson and M. Zembala, Br. Med. Bull., 32, 158 (1976).
4. F. H. Bach and N. K. Vojnow, Science, 153, 545 (1966).
5. M. Doyle, D. E. Parks, and W. Weigle, J. Immunol., 117, 1152 (1976).
6. D. Eardly and R. Bardowell, Fed. Proc., 33, 721 (1974).
7. D. Eardly and R. Gershon, J. Immunol., 117, 313 (1976).
8. H. Folch and B. Waksman, J. Immunol, 113, 127 (1975).
9. R. K. Gershon, Transplant. Rev., 26, 170 (1975).
10. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
11. D. Kastner and D. Rich, Fed. Proc., 35, 571 (1976).
12. S. Katz, D. Parker, G. Sommer, et al., Nature (London), 248, 612 (1974).
13. D. Peavy and C. Pierce, Fed. Proc., 39, 721 (1974).
14. L. Polak and J. Turk, Nature (London), 249, 654 (1974).
15. A. Schwartz, S. Orbach-Arbouys, P. Askenase, et al., Fed. Proc., 35, 571 (1976).
16. T. Takemori and T. Tada, J. Exp. Med., 142, 1241 (1975).
17. J. L. Turk and L. W. Poulter, Clin. Exp. Immunol., 10, 285 (1972).
18. R. Whistler and J. Stobo, J. Exp. Med., 144, 398 (1976).
19. M. Zembala and G. Asherson, Clin. Exp. Immunol., 23, 554 (1976).