LITERATURE CITED

1. T. V. Abramenko, I. V. Vinogradov, V. A. Kabanov, et al., Zh. Mikrobiol., No. 11, 86 (1983).
2. I. V. Vinogradov, V. A. Kabanov, M. I. Mustafaev, et al., Dokl. Akad. Nauk SSSR, 263, No. 1, 228 (1982).
3. V. A. Kabanov, M. I. Mustafaev, A. Sh. Norimov, et al., Dokl. Akad. Nauk SSSR, 243, No. 5, 1330 (1978).
4. V. A. Kabanov, M. I. Mustafaev, V. V. Goncharov, et al., Dokl. Akad. Nauk SSSR, 250, No. 6, 1504 (1980).
5. V. A. Kabanov, R. V. Petrov, and R. M. Khaitov, Zh. Vses. Khim. Obshch. im. D. I. Mendeleeva, 27, No. 4, 417 (1982).
6. V. A. Kabanov, M. I. Mustafaev, A. V. Nekrasov, et al., Dokl. Akad. Nauk SSSR, 274, No. 4, 998 (1984).
7. V. A. Kabanov, M. I. Mustafaev, V. P. Evdakov, and A. D. Antipina, Mol. Biol., 11, 582 (1977).
8. V. A. Kabanov and M. I. Mustafaev, Vysokomol. Soed., 231, 225 (1981).
9. V. A. Kabanov, M. I. Mustafaev, V. V. Belova, and V. P. Evdakov, Biofizika, No. 5, 789 (1978).
10. V. A. Kabanov, M. I. Mustafaev, and V. V. Goncharov, Vysokomol. Soed., 231, 261 (1981).
11. R. V. Petrov, R. M. Khaitov, A. Sh. Norimov, et al., Dokl. Akad. Nauk SSSR, 248, No. 5, 1260 (1979).
12. R. V. Petrov, R. M. Khaitov, and R. I. Ataullakhanov, Immunogenetics and Artificial Antigens [in Russian], Moscow (1983).
13. A. G. Boyes and U. P. Strauss, J. Polymer Sci., 22, 463 (1956).
14. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963)

EXPERIMENTAL ANALYSIS OF THE IMMUNOSTIMULATING PROPERTIES OF VITAMIN A

K. D. Pletsityi

UDC 612.017.1.015.6:577.161.11+615.356:577.161.11/.015.46:612.017.1-063

KEY WORDS: vitamin A; immune response; T suppressors.

Administration of vitamin A to mice undergoing simultaneous immunization with γ-G-globulin, to which they were tolerant, abolishes this tolerance and induces antibody production, i.e., it has the action of an immunologic adjuvant [8]. Subsequently investigations were published in which administration of vitamin A to animals stimulated the immune response to certain T-dependent antigens [5, 6]. Meanwhile some investigators not only were unable to detect any stimulation of the immune response after administration of vitamin A, either *in vitro* or *in vivo*, but in some cases they even observed the development of immunosuppression in such cases [7, 13]. The effect of vitamin A on cellular immunologic reactions has been studied mainly on a model of skin transplantation and blast transformation of lymphocytes in response to the mitogenic stimulation with simultaneous addition of vitamin A to the culture. The results of these investigations were quite contradictory [3, 7, 14, 15].

This paper describes an experimental study of the effect of vitamin A on the T-dependent and T-independent humoral immune response with analysis of immunoregulatory activity of suppressor lymphocytes — cells responsible for regulation of the immune response. The effect of administration of vitamin A to the animals on the intensity of blast transformation of lymphocytes during mitogenic stimulation also was studied.

Laboratory for the Study of Nonspecific Resistance and Immunity, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 600-602, November, 1985. Original article submitted April 8, 1985.

0007-4888/85/0011-1569$09.50

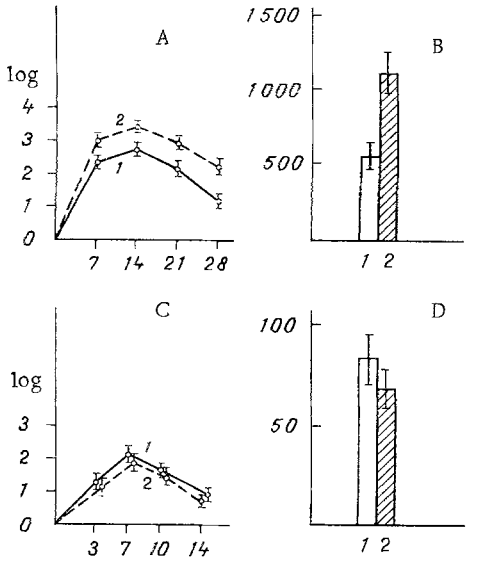

Fig. 1. Effect of vitamin A on production of AFC and antibodies to SRBC (A, B) and Vi-antigen (C, D) in mice (B, D) and rabbits (A, C). Abscissa, days after immunization; ordinate: A, C) antibody titers (log), B, D) number of AFC per 10^6 splenocytes. 1) Control, 2) experiment.

EXPERIMENTAL METHOD

Experiments were carried out on 33 male Chinchilla rabbits weighing 2.5-3 kg and on 220 male CBA mice weighing 16-18 g. In all the experiments the rabbits received intramuscular injections of an oily solution of vitamin A (retinol acetate) in a dose of 200,000 IU daily for 3 days. The vitamin was given to the mice orally in a daily dose of 3000 IU. Animals of the control groups received injections of the oily solvent of vitamin A in the same volume and by the same schedule. Rabbits and mice were immunized 24 h after the final injection of vitamin A with T-independent (Vi-antigen) or T-dependent (sheep's red blood cells, SRBC) antigens. Mice were immunized intraperitoneally with 0.5 ml of a 5% suspension of SRBC or 1 μg of crystalline Vi-antigen, diluted in 0.2 ml of physiological saline. After 4 days the number of antibody-forming cells (AFC) in the animals' spleen was determined by Jerne's direct and indirect methods [10, 11] respectively. Rabbits were immunized with SRBC (2 ml of a 20% suspension, intraperitoneally) or with Vi-antigen (100 μg intravenously), after which titers of hemagglutinating antibodies were determined in the serum by the passive hemagglutination test (PHT) at different times after immunization.

The effect of vitamin A on generation of antigen-specific suppressor cells was studied by the method described previously [1]: After receiving vitamin A or oil, mice were immunized with SRBC, and 2 weeks later $50 \cdot 10^6$ splenocytes were injected intravenously into intact mice, which were immunized simultaneously with SRBC. Control mice received splenocytes from unimmunized animals. Jerne's test was then carried out. The level of antigen-specific suppression was expressed as a percentage of the control. To detect suppressor cells induced by concanavalin A (Con A) 10^6 splenocytes from mice receiving vitamin A or the solvent were incubated for 48 h in medium RPMI-1640 (Flow Laboratories, USA) with the addition of antibiotics, 10% embryonic calf serum (Flow, USA) and Con A (20 μg per 10^6 cells, from Serva, West Germany). The reaction was then stopped by the addition of mitomycin C (incubation for 30 min at 37°C, mitomycin C in a dose of 40 μg/10^6 cells, from Serva). The cells were washed thoroughly to remove mitomycin C, 10^6 splenocytes were added, and the suspension of cells was cultured for 72 h in the presence of phytohemagglutinin (PHA, 20 μg/10^6 cells, from Serva). To each sample 1 μCi of ^{3}H-thymidine was added 6 h before the end of culture. Blast transformation of the lymphocytes in response to their mitogenic stimulation by PHA or Con A was determined in the same way: Spleen cells from animals treated beforehand with vitamin A or the oily solvent were cultured for 3 days with the mitogens, which were used in a dose of 20 μg/10^6 cells, after which thymidine was added and radioactivity counted. The results were subjected to statistical anlysis by Student's t test.

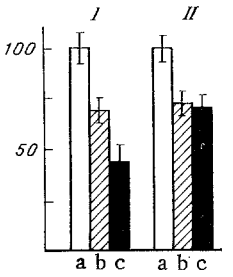

Fig. 2. Effect of vitamin A on generation of antigen-specific and antigen-nonspecific suppressors in mice. Ordinate, % of control, taken as 100. I) Number of AFC in response to SRBC (antigen-specific suppression, suppressor cells induced by injection of the same antigen); II) incorporation of ^{3}H-thymidine into lymphocytes stimulated by PHA (antigen-nonspecific suppression, suppressor cells induced by the action of Con A *in vitro*): a) in absence of suppressor cells; b) after addition of suppressor cells from animals receiving vitamin A; c) after addition of suppressor cells from animals receiving oil used as solvent for vitamin A.

EXPERIMENTAL RESULTS

Data showing the effect of vitamin A on the immune response to SRBC and Vi-antigen are given in Fig. 1. The use of vitamin A caused marked stimulation of AFC and antibodies against T-dependent antigen (SRBC), whereas no effect of vitamin A could be detected when T-independent Vi-antigen was used. The effect of vitamin A on generation of antigen-specific and antigen-nonspecific suppressors is shown in Fig. 2. Transfer of splenocytes from animals immunized with SRBC after previously receiving vitamin A, into intact mice caused significantly weaker suppression of the immune response ($P < 0.01$) than transfer of splenocytes from control animals, immunized after administration of the oily solvent only. Meanwhile vitamin A had no significant effect on activity of cells responsible for nonspecific suppressor functions. A study of blast transformation of lymphocytes to PHA and Con A revealed no significant effect of vitamin A (data not given).

The results thus showed that vitamin A stimulates the humoral immune response to a T-dependent antigen (SRBC) but does not affect the T-independent immune response. The system of T helper cells is involved in the realization of the immunostimulant action of vitamin A. Helper cells are not required for the T-independent response. This state of affairs explains the absence of immunostimulant action of vitamin A when Vi-antigen was used. Data on the possible involvement of helper cells in the realization of the effects of vitamin A also have been reported by other workers [4, 12]. In the first of these publications thymectomized mice and mice irradiated and restored with bone marrow cells lost their ability to demonstrate enhancement of the immune response to T-dependent antigen under the influence of vitamin A. It has recently been shown [12] that vitamin A stimulates the intensity of the graft versus host reaction, and that the cells responsible for this stimulation have the phenotype of T helper cells. However, we know that helper cells are under the regulatory control of T suppressor [2, 9]. The results of the present experiments confirm that the original target for the action of vitamin A is a subpopulation of cells which perform an antigen-specific suppressor function. Vitamin A inhibits their suppressor activity, as is shown by stimulation of helper cells and, ultimately, by intensification of the T-dependent immune response.

The results of the present investigation are in full agreement with data in the literature showing that the immune response to T-dependent antigens is controlled by antigen-specific suppressors, whereas the response to T-independent antigens is controlled by antigen-nonspecific suppressors [2]. In the present experiments inhibition of activity of antigen-specific suppressors by vitamin A was accompanied by stimulation of the immune response to SRBC. Meanwhile vitamin A did not affect the generation of antigen-nonspecific suppressors, and as a result there was no change in the response to Vi-antigen.

LITERATURE CITED

1. V. M. Pisarev and L. A. Pevnitskii, Byull. Éksp. Biol. Med., No. 5, 571 (1977).
2. R. M. Khaitov, in: Progress in Socience and Technology. Series: Oncology [in Russian], Vol. 13, Moscow (1984), pp. 5-45.
3. J. Abb and J. Deinhardt, Exp. Cell Biol., 48, 169 (1980).
4. A. G. Allison and A. J. S. Davies, Nature, 233, 330 (1971).
5. J. B. Barnett, Int. Arch. Allergy, 22, 227 (1983).
6. B. E. Cohen and I. K. Cohen, J. Immunol., 111, 1376 (1973).
7. G. Dennert and R. Lotan, Eur. J. Immunol., 8, 23, (1978).
8. D. W. Dresser, Nature, 217, 527 (1968).
9. D. R. Green, P. M. Flood, and R. K. Gershon, Ann. Rev. Immunol., 1, 439 (1983).
10. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
11. J. P. Lake, N. D. Reed, J. T. Ulrich, et al., Immunol. Comm., 6, 149 (1977).
12. M. Malkovsky, A. J. Edwards, R. Hunt, et al., Nature, 302, 338 (1983).
13. A. A. McColm, R. Bomford, and L. Dalton, Parasit. Immunol., 4, 337 (1982).
14. P. B. Medawar and R. Hunt, Immunology, 42, 349 (1981).
15. J. P. Neifeld, H. M. Lee, and N. E. Hutcher, J. Surg. Res., 19, 225 (1975).