

IMMUNE RESPONSE OF REGIONAL LYMPH GLAND CELLS AGAINST THE BACKGROUND OF 5-HYDROXYTRYPTOPHAN AND 3,4-DIHYDROXYPHENYLALANINE ACTION

L. V. Devoino and L. S. Eliseeva (Korovina)

UDC 612.428:612.017.1)014.46

Experiments on mice immunized with bovine serum albumin showed that injection of 5-hydroxytryptophan decreases, while injection of 3,4-dihydroxyphenylalanine increases the number of antibody-forming cells by comparison with the control. 5-Hydroxytryptophan also reduces the quantity of antigen adsorbed by antibody-producing cells.

* * *

Injection of serotonin and its precursor 5-hydroxytryptophan into animals lowers the titer of humoral antibodies and inhibits the development of hypersensitivity of delayed type. Injection of 3,4-dihydroxyphenylalanine stimulates antibody production to some degree, as shown by an increase in the titers of antibodies in the initial period of observation and the earlier attainment of the highest possible intensity of immunity [1]. However, the results of these investigations have not revealed the cell changes with which the observed effect is associated.

The object of this investigation was to study antibody production by single cells of regional lymph glands in animals receiving 5-hydroxytryptophan and 3,4-dihydroxyphenylalanine.

EXPERIMENTAL METHOD

The method used was that suggested by Shvartsman [3], based on adsorption of erythrocytes conjugated with antigen through bisdiazotized benzidine, on the surface of the immunologically active cell. This method enables the production of antibodies against soluble protein antigens by single cells to be determined.

Experiments were carried out on 147 male mice of lines C57BL/6 and CBA, aged 3-6 months and weighing 20-30 g. The animals were divided into 17 groups, with 8-10 mice in each group. Crystalline bovine serum albumin (BSA) was used as antigen and was injected as a single dose of 2 mg per mouse together with Freund's complete adjuvant, subcutaneously into the upper third of both thighs, 0.1 ml of the mixture being injected into each thigh. On the 5th day the regional lymph glands were removed and a suspension of single cells made from them [2]. The suspension of washed cells, mixed with a 5% suspension of sheep's erythrocytes conjugated with the antigen, was incubated for 15 min at 37°. Previously synthesized bisdiazotized benzidine was used for conjugation of the BSA with the erythrocytes [4]. Examination under the microscope was carried out in a moving system with floating cover glass, and using an immersion phase-contrast optical system. In each test at least 1000 cells were examined, and the number of antibody-producing cells and the number of erythrocytes adsorbed by them were determined. The adhesion index (the mean number of erythrocytes adsorbed by one producer cell) was calculated.

The DL-5-hydroxytryptophan (5-HTP) was manufactured by the firm of Reanal (Hungary) and the DL-3,4-dihydroxyphenylalanine (DHPA) by the firm GMBH and Co. (West Germany).

Laboratory of Physiology of Immunity, Institute of Physiology, Siberian Division, Academy of Sciences of the USSR, Novosibirsk. (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. 69, No. 2, pp. 63-66, February, 1970. Original article submitted April 2, 1969.

©1970 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for \$15.00.

TABLE 1. Antibody Production by Cells of Lymph Glands from Control Mice

Group	Cells	Number of antibody-forming cells*(M±m)	Titers of serum anti-bodies in cell donors
1	Of intact animals	0.4±0.2	0
2	Of intact animals treated with antiserum	0.4±0.2	0
3	Of animals receiving anti-serum intraperitoneally	0.5±0.2	1:10 240
4	Of immunized animals	4.6±0.4	0

*Per thousand cells examined.

TABLE 2. Effect of 5-HTP on Antibody Production by Single Lymph Gland Cells

Line and age of mice	Group	Dose of compound (in mg/kg)	Method and times of injection*	Number of antibody-forming cells (M±m)	P	Adhesion index (M±m)	P
CBA 3.5-4 months	1	Control	—	4.6±0.4	—	1.5±0.3	—
	5	50	Intraperitoneally, once on day of immunization	4.8±0.6	> 0.5	2.6±0.9	> 0.2
	6	50	Intraperitoneally, daily from day -2 to day +4	2.6±0.5	< 0.01	1.6±0.5	> 0.5
	7	50	Subcutaneously with adjuvant on day of immunization	1.5±0.5	< 0.001	1.7±1.0	> 0.5
C57BL/6 2.5-3.5 months	8	Control	—	7.6±0.4	—	2.0±0.2	—
	9	100	Intraperitoneally, daily from day -2 to day +4	3.9±0.5	< 0.001	1.2±0.1	< 0.01

*Here and in Table 3 the sign — denotes days before immunization, the sign + days after immunization.

EXPERIMENTAL RESULTS

To detect the specificity of the reaction, control experiments were carried out on the immune animals (Table 1) with cells of lymph glands taken from: 1) intact animals (group 1); 2) intact animals but the cells subsequently treated with an isologous antiserum (group 2); 3) passively immunized animals receiving an injection of 0.4 ml isologous antiserum with a titer of anti-BSA antibodies of 1:320,000 24 h beforehand (group 3). The results given in Table 1 show that nonspecific adsorption was 10 times less than specific adsorption in the immune animals.

Analysis of the data obtained after injection of 5-HTP into the mice (Table 2) shows that a single intraperitoneal injection of a dose of 50 mg/kg in saline solution simultaneously with antigen caused no changes either in the number of producer cells or in the adhesion index (group 5) compared with the corresponding control values (group 1).

TABLE 3. Effect of DHPA on Antibody Production by Single Cells of Lymph Glands from CBA Mice

Age of mice (in months)	Group	Dose of compound (in mg/kg)	Method and time of injection	Number of antibody-forming cells ($M \pm m$)	P	Adhesion index ($M \pm m$)	P
3-3.5	1 10	Control 100	— Daily, intraperitoneally, from -2 to day + 4	4.6 \pm 0.4	—	1.5 \pm 0.3	—
				4.6 \pm 0.7	—	2.4 \pm 0.8	< 0.1
6-6.5	11	Control	—	3.5 \pm 0.4	—	2.5 \pm 0.6	—
	12	100	Once, intraperitoneally, on day of immunization	3.6 \pm 0.3	> 0.5	2.5 \pm 0.6	> 0.5
	13	100	Daily, intraperitoneally, from day -2 to day + 4	5.4 \pm 0.5	< 0.05	1.9 \pm 0.4	0.2
3-3.5	14 15	Control 100	— Daily, intraperitoneally, from day -2 to day + 4	5.6 \pm 0.7	—	1.2 \pm 0.2	—
				5.5 \pm 1.0	> 0.5	1.1 \pm 0.1	> 0.5
6-6.5	16 17	Control 100	— Daily, intraperitoneally, from -2 to day + 4	4.0 \pm 0.7	—	1.8 \pm 0.9	—
				0.4 \pm 0.7	< 0.05	2.0 \pm 0.4	> 0.5

Repeated injections of 5-HTP were started two days before immunization and ended on the 4th day after immunization, 24 h before the tests were carried out. After repeated injection of the same dose of 5-HTP (group 6) a marked decrease was observed in the number of cells participating in the immune response. The most marked decrease in the number of active cells was observed following a single subcutaneous injection of 5-HTP in a dose of 50 mg/kg, mixed with Freund's adjuvant (group 7). The gradual release of 5-HTP from the depot of adjuvant evidently prolonged the period of increased serotonin concentration compared with that observed after a single injection of the compound made up in saline solution, and the continuous liberation provided better conditions than repeated injections of the same dose at 24 h intervals.

The intensity of activity of the producer cells following a single intraperitoneal injection of 50 mg/kg 5-HTP remained at the control level. An increase in the dose of the compound to 100 mg/kg, if it was given repeatedly (Table 2, group 9), not only reduced the number of producer cells, but also lowered the adhesion index of these cells.

The results obtained by injection of DHPA into the mice (Table 3) showed that a single injection of 100 mg/kg of the compound on the day of immunization had no effect on the immune response (group 12). So far as repeated injections were concerned, the effect of DHPA depended on the age of the animals, which was not true of the effect of 5-HTP. In young mice (aged 3-3.5 months), for instance, no difference was found in the number of antibody-forming cells or in the number of erythrocytes adhering to them between the control and experimental animals (group 10), while in older mice (6-6.5 months) an increase in the number of producer cells was found (group 13). This fact was also confirmed in an analogous experiment (groups 15 and 17).

Hence, after injection of 5-HTP into mice, a decrease in the number of antibody-producing cells was observed, and if the dose was increased, the number of erythrocytes adherent to the cell also was reduced. This could indicate either a decrease in the intensity of antibody production by the cell or a change in character of the antibodies detected in these experiments. Stimulation of the immune response following administration of DHPA is associated with an increase in the number of producer cells.

LITERATURE CITED

1. L. V. Devoino, L. S. Korovina, and R. Yu. Ilyutchenok, *Europ. J. Pharmacol.*, 4, 441 (1968).
2. Ya. S. Shvartsman, *Byull. Éksperim. Biol. i Med.*, No. 12, 88 (1964).
3. Ya. S. Shvartsman, *Byull. Éksperim. Biol. i Med.*, No. 12, 75 (1966).
4. J. Cordon, B. Rose, and A. H. Sehon, *J. Exp. Med.*, 108, 37 (1958).