DYNAMICS OF FORMATION OF γM- AND γG-ANTIBODIES IN MICE AFTER ADMINISTRATION OF SEROTONIN AND ITS PRECURSOR 5-HYDROXYTRYPTOPHAN

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Administration of serotonin and its presursor 5-hydroxytryptophan into C57BL/6 and BALB/c mice immunized with bovine serum albumin lengthened the latent period of synthesis of γG-antibodies and, in some experiments, of γM-antibodies also, lowered the liters of γM-antibodies, and delayed the attainment of their maximum. After administration of serotonin by some methods, the duration of synthesis of γM-antibodies was reduced. The most marked inhibition of synthesis of γM- and γG-antibodies was observed after administration of serotonin in Freund's incomplete adjuvant.

An increase in the serotonin level in an immunized animal inhibits humoral antibody formation. This effect is observed after administration of the biogenic amine itself [1] and also of substances affecting its metabolism [2, 9]. However, the changes in the main classes of antibodies taking place under these circumstances are not known.

The object of the present investigation was to study the dynamics of formation of γM- and γG-antibodies during the primary immune response in animals receiving serotonin and its precursor 5-hydroxytryptophan (5-HTP).

EXPERIMENTAL METHOD

Experiments were carried out on 356 male inbred mice of lines C57BL/6 and BALB/c aged 4 months and weighing 20-25 g.

The antigen used was crystalline (Difco) bovine serum albumin (BSA), which was injected in a dose of 5 mg/kg body weight intraperitoneally in 0.2 ml physiological saline into BALB/c mice and subcutaneously in 0.2 ml Freund's complete adjuvant (0.1 ml on each side) into C57BL/6 mice. Blood was collected from the retro-orbital sinus into the same test tube from 15-20 animals before immunization and 4, 7, 10, 14, 21, 32, and 40 days after immunization.

The γM- and γG-antibodies were separated by gel-filtration of 0.4 ml serum through Sephadex G-200 on a 2.3 × 60.0 cm column, using buffer with pH 8.0 (0.02 M Tris-HCl plus 0.28 M NaCl solution) [3].

The protein concentration in each sample, collected by a fraction collector (Radi Rac Sweden) at the rate of 5.0-6.5 ml/h in a volume of 2.5 ml, was determined on a VS4-2P spectrophotometer at 280 μm. Samples with the maximal protein concentration were mixed in peak I (γM-antibodies) and in peak II (γG-antibodies), and then diluted with buffer solution to concentration equivalent to 1/20 or1/40 of the original serum.

Activity of the antibodies in these samples and in the whole serum before and after contact with 2-mercaptoethanol (2-ME), which selectively inactivates molecules of macroglobulins by rupturing disulfide

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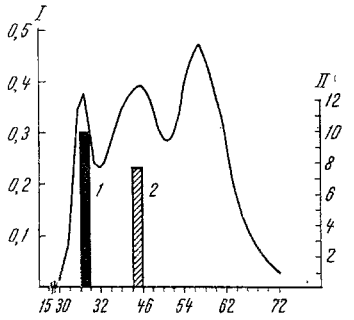

Fig. 1

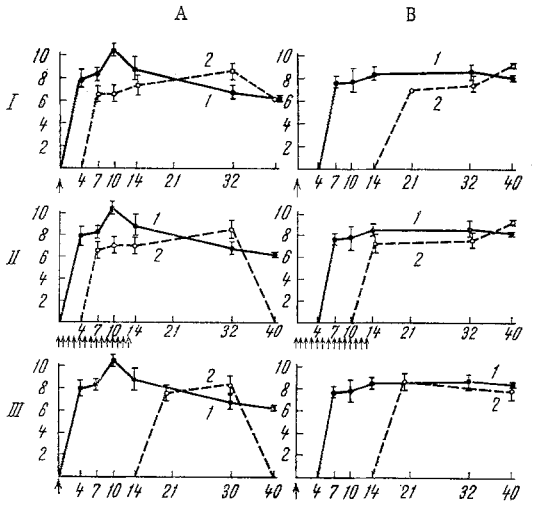

Fig. 2

Fig. 1. Fractionation of serum on Sephadex G-200. Serum of control BALB/c animals on 10th day after immunization: 1) ME-sensitive antibodies; 2) ME-resistant antibodies. Abscissa, Fraction No.; ordinate: i) optical density at 280 μm, ii) titer of antibodies (in $\log_2$).

Fig. 2. Primary immune response in BALB/c mice (immunization with bovine serum albumin, 5 mg/kg, intraperitoneally): A) γM-antibodies; B) γG-antibodies. 1) Titers of antibodies in control animals; 2) titers of antibodies in animals with raised serotonin level. I) Injection of 5-HTP, 100 mg/kg, in Freund's incomplete adjuvant, subcutaneously. II) Injection of serotonin, 50 mg/kg, in physiological saline, subcutaneously; III) Injection of serotonin, 50 mg/kg, in Freund's incomplete adjuvant, subcutaneously. Arrow indicates injection of compound. Abscissa, days after immunization; ordinate, titer of anitbodies (in $\log_2$).

bonds [8], was determined by Boyden's passive hemagglutination method [7]. The serum was treated with a 0.2 M solution of 2-ME in phosphate buffer, pH 7.2, for 24 h at room temperature. DL-5-hydroxytryptophan (HTP, Reanal, Hungary) and serotonin (5-hydroxytryptamine) creatinine-sulfate, containing 43.47 mg serotonin base per 100 mg (Gee Lawson Chemicals Ltd., England), were used in the investigation. The animals of series I (C57BL/6) received 5-HTP in a dose of 200 mg/kg in physiological saline intraperitoneally on the day of immunization and for 8 days thereafter, and the mice of series II (C57BL/6) received 5-HTP in a dose of 100 mg/kg in Freund's incomplete adjuvant by subsutaneaus injection on the day of immunization and for 4 days thereafter; the mice of series III (BALB/c) received 5-HTP in a dose of 100 mg/kg in Freund's incomplete adjuvant on the day of immunization; the animals of series IV (BALB/c) received serotonin in a dose of 50 mg/kg in physiological saline subcutaneously on the day of immunization and for 13 days thereafter; the five mice of series V (BALB/c) received serotonin in a dose of 50 mg/kg in Freund's incomplete adjuvant subcutaneously on the day of immunization. Instead of the compound, the control animals received an injection of the corresponding volume of physiological saline or adjuvant.

EXPERIMENTAL RESULTS

Three protein peaks were obtained from each serum by gel filitration. Treatment of the peak 1 fraction with 2-ME led to complete loss of activity of the antibodies, while in peak 2 there was no change (Fig. 1), indicating that the peak 1 fraction contained ME-sensitive antibodies (γM) and that the peak 2 fraction contained ME-resistant antibodies (γG).

Analysis of the γM- and γG-antibodies from animals of the control series showed that γM-antibodies appeared in BALB/c mice on the 4th day and reached their highest level on the 10th day, while in C57BL/6 mice the latent period of formation of γM-antibodies were 7 days, the maximum occuring on the 14th day. In both groups γG-anitbodies appeared 3 days later than the γM-anitbodies, in agreement with results indicating the order antibody synthesis [6, 17].

Injection of 5-HTP and serotonin in all series of experiments lowered the level of γM-antibodies principally at the time of the maximal increase in antibody formation until the 32nd day. The inhibitory effect of the compounds was most marked in the BALB/c mice, in which the latent period was also prolonged to 7-21 days (4 days in the control) and the duration of the response was reduced depending on the method used to raise the blood serotin level (Fig. 2A).

The effect of injection of 5-HTP and serotonin on the formation of γG-antibodies in mice of both lines was manifested as lengthening of the latent period to 14-21 days, after which the antibody titers reached the control level (Fig. 2B). Attainment of the maximum of antibody formation by the antibodies of this type in series III and IV was delayed until the 40th day (Fig. 2B: I, II).

Continuous liberation of serotonin in small portions as the result of its injection in Freund's incomplete adjuvant led to a more marked inhibitory effect on the formation of both types of antibodies (Fig. 2A: III, B: III).

Inhibition of the primary immune response, which was most marked in the initial period after antigenic stimulation, was thus connected with lengthening of the latent period and a decrease in the synthesis of γM-antibodies and prolongation of the latent period of formation of the γG-antibodies.

These results, indicating that synthesis of γM-antibodies is independent of the synthesis of γG-antibodies in animals with a raised serotonin level and that their sensitivity to serotonin differs slightly are more in agreement with the hypothesis that two different cells lines synthesize γM- and γG- antibodies [15, 16] than with the hypothesis that the same cell population synthesizes first the γM- and then the γG-antibodies [14]. This suggests that the two lines of cells respond differently to serotonin, and that those synthesizing γG-antibodies give a more marked response than those synthesizing γM-antibodies. There is evidence in the literature of differences in the sensitivity of γM- and γG-antibodies to x-ray irradiation [13] and to certain chemicals [6, 12, 15].

The mechanism by which the intensity of antibody synthesis is lowered by serotonin is not yet known. It could perhaps be connected with the property of serotonin of inhibiting cell mitoses [4], and since active proliferation is probably necessary for the synthesis of γG-antibodies whereas it is not essential at all or is less necessary for γM-antibodies [10], this will account for the greater effect of serotonin on the formation of γG-antibodies. The possibility likewise cannot be ruled out that serotonin somehow reduces the uptake of antigen by macrophages or reduces its content in germinal centers, thereby reducing the synthesis of both types of antibodies, possibly by different degrees. Experiments have shown [11, 18], that the dose of antigen and the length of its survival in the body are factors which regulate the relative proportions of γM- and γG-antibodies.

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