MICROBIOLOGY AND IMMUNOLOGY

Vasopressin: Secondary Immune Response and Antigen-Specific Suppressors

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A single injection of arginine vasopressin during primary immunization stimulates secondary immune response. Vasopressin decreases the activity of antigen-specific suppressors induced by hyperimmune doses of the antigen.

Key Words: *vasopressin; secondary immune response; antigen-specific suppressors*

The immunoregulating role of vasopressin (VP) has been recently revealed and VP receptors detected on immunocompetent cells [6,7]. Vasopressin stimulates phagocytosis [3], antibody production [2,5], and the production of bioactive substances in immunocytes [10]. It is produced in the thymus [8] and is present in splenic lymphocytes [9]; its release is stimulated by interleukins [11]. The immunomodulating effect of VP is little known. Vasopressin probably affects immunological memory and tolerance. Previously we showed that a single injection of VP immediately after immunization of mice with sheep erythrocytes (SE) increases the number of antibody-producing cells (APC) in the spleen at the peak of immune response [2]. This observation prompted the investigation in a probable effect of VP on secondary immune response and functional activity of antigen-specific suppressors.

MATERIALS AND METHODS

Experiments were carried out on male C57BL/6 mice. The animals were immunized in the optimal immunogenic dose of 5×10^8 and hyperimmune dose of 3×10^9 SE in 0.2 ml normal saline intraperitoneally.

The immune response was evaluated by the level of IgM- and IgG-APC in the spleen. The spleen was isolated under ether narcosis and homogenized in 2 ml medium 199. The APC content was determined by direct and indirect local hemolysis in Canningham's modification [1]. For indirect measurements, the optimal dilution of purified rabbit antibodies to murine IgG (Calbiochem) in the was used. The titer of serum hemagglutinins was determined.

Specific suppression of immune response by splenocytes was assessed in the adoptive transfer system [4]. Donor splenocytes were isolated as described previously, pooled suspensions of donor cells were washed twice in medium 199 with antibiotics and injected intraperitoneally to intact mice in a dose of 5×10^7 cells/animal, which were immunized after 30 min with SE in the optimal immunogenic dose. The immune response was evaluated on day 5 after immunization.

Arginine-VP (Sigma) was used.

The results were processed using Student's t test.

RESULTS

The effect of VP on induction of antigen-specific suppression of immune response was studied in the first series of experiments. Three groups of C57BL/6 mice were splenocyte donors: 1) intact, 2) hyperimmunized with SE, and 3) hyperimmunized with SE+VP, 1 μg. Splenocytes were isolated 2 weeks after hyperimmunization. The recipients were divided into 4 groups: 1) administered splenocytes from intact donors; 2) spleno-

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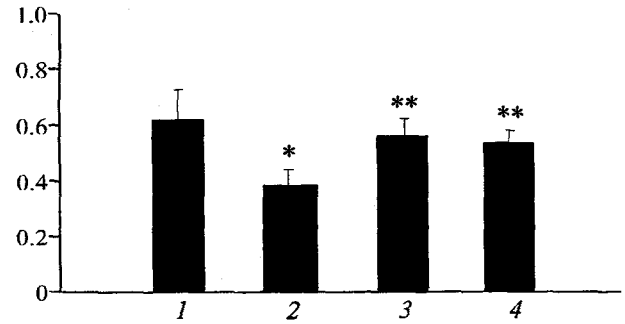

Fig. 1. Effect of vasopressin on the activity of antigen-specific suppressors. Ordinate: number of IgM-antibody-producing cells in the spleen of recipients on day 5 after immunization with sheep erythrocytes ($\times 10^5$ cells). Recipient animals were subcutaneously injected with normal saline and intact donor splenocytes (*1*), splenocytes of hyperimmune donors (*2*), splenocytes of donors hyperimmunized after injection of arginine-vasopressin (*3*), and arginine-vasopressin (1 μg) and splenocytes of hyperimmune donors (*4*). $p<0.05$: **vs. 1*; ***vs. 2*.

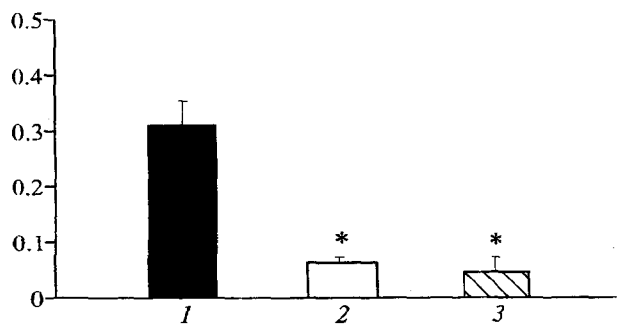

Fig. 2. Effect of a single injection of vasopressin (together with primary immunization) on the antigen-specific suppressor activity of splenocytes isolated during the formation of secondary immune response. Ordinate: count of IgM-antibody-producing cells in recipient's spleen on day 6 after immunization with sheep erythrocytes ($\times 10^5$ cells). Recipient animals were transferred intact donor splenocytes (*1*), splenocytes isolated during development of secondary immune response in animals injected with normal saline (*2*) or arginine-vasopressin (*3*) during the first immunization. *$p<0.001$ *vs.* intact donors.

cytes from group 2 donors; 3) splenocytes from group 3 donors; and 4) splenocytes of group 2 donors+VP, 1 μg. All experimental mice were subcutaneously injected with 0.2 ml normal saline. The immune response of the recipients was assessed on day 5 after immunization. The results showed a VP-induced decrease in the splenocyte suppressor activity, caused by hyperimmune doses of the antigen (Fig. 1). The immune response of recipients of splenocytes from hyperimmune donors injected with normal saline was 62% of the immune response of control animals (Fig. 1, *2, vs. 1*). Splenocytes of the donors injected with VP after the tolerogenic dose of SE suppressed the production of IgM-APC in the recipient spleen only by 91% of the control (Fig. 1, *3 vs. 1*), and the immune response of these animals was significantly higher than in animals injected with normal saline (Fig. 1, *3 vs.* 2). Vasopressin stimulated the immune response if it was injected to recipients after splenocytes rich in antigen-specific suppressors (Fig. 1, *4 vs.* 2). Thus, the anti-tolerogenic and immunostimulating effects of VP were observed as antigen-specific suppressor activity after VP injection to donors and to recipients after a tolerogenic dose of immunocytes.

In the second series of experiments we studied the effect of VP on secondary immune response. Two groups of animals were immunized twice at a 28-day interval by SE in the optimal immunogenic dose. Group 1 animals were injected subcutaneously with 0.2 ml normal saline immediately after the first immunization. Group 2 animals were injected with VP (1 μg in 0.2 ml normal saline) simultaneously with immunization. The immune response was evaluated by the counts of IgM- and IgG-APC in the spleen on day 5 after the second immunization. The sum of IgM- and IgG-APC in the spleen of animals injected with VP was significantly ($p<0.01$) higher (2.70±0.11) than in animals injected with normal saline alone (1.51± 10.29). These results indicate that VP stimulates the processes associated with secondary immune response, i.e., immunological memory. Hemagglutinin titers (expressed in -ln) at the same intervals were virtually the same: 7 in animals treated with VP and 6.3 in the controls.

In the third series of experiments we investigated the probable role of changes in antigen-specific suppressor activity in VP stimulation of secondary immune response. Splenocytes of animals from the second series of experiments in a dose of 5×10^7 cells/mouse were transferred to intact recipients which were immunized after 0.5 h with the optimal immunogenic dose of SE. The immune response was evaluated by the titers of serum hemagglutinins and the counts of IgM-APC in the spleen on day 6 after immunization. Splenocyte transfer from experimental animals immunized twice and from controls under these conditions caused a strong reaction to SE (evaluated by serum hemagglutinin titers) in the recipients, which was comparable to that in animals transferred intact donor splenocytes. The titers of hemagglutinins in the recipients of splenocytes from the donors treated with VP or normal saline together with the first immunization were 5.5 and 5.9, respectively, *vs.* 2.9 in the recipients of intact donor splenocytes ($p<0.05$). The formation of splenic IgM-APC was suppressed in the recipients of splenocytes from donors immunized twice in comparison with those transferred splenocytes from intact animals, irrespective of whether they were injected with VP or normal saline during the first immunization (Fig. 2). The total antigen-specific suppressor activity of experimental and control animals was virtually the same, and changes in the secondary immune response under the effect of VP probably do not de-

pend on it, in contrast to the mechanisms involved in the VP stimulation of primary immune response.

These results show the role of VP in the mechanisms of immunological memory formation within the framework of neuroimmune reactions.

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