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EFFECT OF CHORIONIC GONADOTROPHIN ON COOPERATION BETWEEN SPLENOCYTES FORMING THE PRIMARY IMMUNE RESPONSE

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Chorionic gonadotrophin (CG) is one of the principal hormones of reproduction, and successful implantation and development of the semiallogeneic blastocyst depends on its secretion [2]. Production of the hormone is regularly found in cancer cells [15], and from this standpoint CG has been regarded as a factor preventing rejection of foreign tissue [1]. In all probability the hormone has an immunoregulatory action, but no clear ideas regarding the mechanism of this action have been formulated.

The aim of this investigation was to study the effect of CG on intercellular cooperation processes during the formation of an adoptive immune response, and also to study the identification of certain mediators of this interaction as possible intermediaries in the regulatory action of the hormone.

EXPERIMENTAL METHOD

Experiments were carried out on mature male (CBA × C57BL/6)F_1 mice weighing 18-20 g. Bilateral ovariectomy was performed on some of the animals under ether anesthesia. The period of postoperative rehabilitation was 4 weeks.

During investigation of the action of CG on cooperation between T and B lymphocytes the model suggested previously [13] was adopted, with fractionated splenocytes.

Simultaneously with the antigen (2×10^8 sheep's red blood cells), 4-6 h after lethal irradiation (219.3 mCi/kg) of the recipient mice, they were given an intravenous injection of 10^7 T cells, isolated by filtration of a splenocyte suspension through nylon wadding [12], and 10^7 B cells, isolated by treatment of a suspension of spleen cells with anti-Br-θ-serum [8] and guinea pig complement. The T lymphocytes were identified in the cytotoxic test with anti-Br-θ-serum [9], and B lymphocytes by the EAC-rosette-formation method [4]. On the 5th day the number of antibody-forming cells (AFC) in the animals' spleen was determined by the method in [11].

In the experiments of series I the recipients were given three injections of CG (Moscow Endocrine Factory) on alternate days subcutaneously in a dose of 40 or 200 U, starting from the moment of cell transfer. To determine whether the effect of the hormone was dependent on prostaglandins (PG), in the experiments of series II, parallel with CG, recipient mice received an injection of Voltaren (Pliva, Yugoslavia), an inhibitor of prostaglandin synthetase, in a dose of 3 mg/kg. Both series of experiments were conducted both on castrated and on noncastrated animals. In all the calculations logarithms of the number of AFC were used.

To determine the effect of different doses of CG on interleukin-2 (IL-2) production by the splenocytes, a cell culture (2×10^6 cells/ml), obtained by homogenization of the spleens of

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TABLE 1. Effect of CG on Immune Response of Irradiated Recipient Mice Receiving a Mixture of Syngeneic T and B Spleen Cells from Intact Donors

Group No.	Treatment of recipients	Number of AFC in spleen
1 (n=17)	Injection of CG 40 U	3710 (3198—4296) $p_{1-3}<0{,}001$; $p_{1-2}<0{,}02$
2 (n=18)	200 U	2143 (1821—2522) $p_{2-3}<0{,}002$
3 (n=34)	Injection of solvent of hormone	1170 (1053—1299)
4 (n=16)	Ovariectomy + injection of CG 40 U	883 (764—1021) $p_{4-6}>0{,}05$; $p_{4-5}<0{,}001$
5 (n=17)	200 U	468 (407—538) $p_{5-6}<0{,}002$
6 (n=17)	Ovariectomy + injection of solvent of hormone	832 (727—951)

Legend. Mean values and confidence intervals are given.

intact female mice, freed from spontaneous suppressor T cells by preincubation for 24 h, was treated with a mitogen (concanavalin A (con A, 5 μg/ml) together with CG in a dose of 20 or 100 U/ml, which corresponded to doses of 40 and 200 U in experiments in vivo. Incubation of the splenocytes with mitogen or with mitogen and CG continued for 4 h, after which the cells were washed twice, resuspended, and incubated for the next 24 h. At all stages of culture medium RPMI-1640 was used with the addition of L-glutamine (2 mM), 2-mercaptoethanol (5×10^{-5} M), and gentamicin (100 μg/ml). The cells were cultured in an atmosphere containing 5% CO_2 at 37°C. The supernatant from the cultures was tested for the presence of IL-2 by the co-stimulation method [10]. DNA synthesis in cultures of syngeneic thymocytes after addition of the supernatant was tested by incorporation of ^{3}H-thymidine (5 μCi/ml, 7 h, 37°C) at the end of 3 days of incubation and expressed as the stimulation index: the ratio of the quantity of ^{3}H-thymidine (in cpm) taken up by the cells in the stimulated cultures and the amount of ^{3}H-thymidine taken up by control cultures.

The results obtained in the experimental models in vivo and in vitro were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Injection of CG into noncastrated animals significantly increased the ability of their lymphoid cells to form an adoptive immune response at the stage of antigen-dependent differentiation; the immunostimulating effect of the hormone, moreover, was inversely proportional to its dose (Table 1). If CG was injected into ovariectomized animals, in a small dose (40 U) it did not affect the formation of the immune response, but in a large dose (200 U) it had the opposite, immunodepressive effect. Considering the ability of CG, in both normal and irradiated recipients, to potentiate the production of female sex steroids [6], and also the immunostimulating action of the latter [3], it can be tentatively suggested that a specific immunodepressive effect for CG is observed in ovariectomized mice. Meanwhile stimulation of the adoptive immune response in noncastrated females is evidently mediated through female sex steroid hormones, whose action completely abolishes CG-dependent immunosuppression.

The antigen-dependent stage of differentiation of the immunocompetent cells forming the primary immune response is a multicomponent and multidimensional system, including interactions

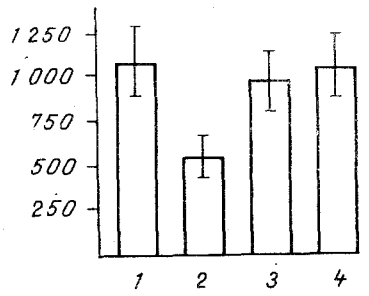

Fig. 1

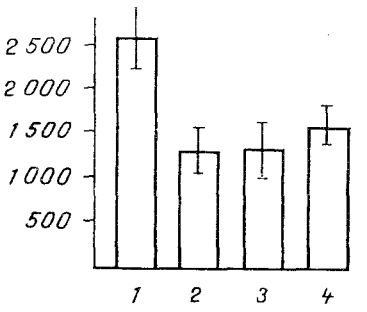

Fig. 2

Fig. 1. Effect of CG on level of adoptive immune response in female mice with intact ovaries, during blocking of endogenous PG synthesis. Ordinate (here and in Fig. 2) — number of AFC in spleen. 1) Injection of solvent of hormone; 2) injection of solvent of hormone and PG-synthetase inhibitor; 3) injection of CG (40 U) and PG synthetase inhibitor; 4) injection of CG (200 U) and of PG-synthetase inhibitor.

Fig. 2. Effect of CG on level of adoptive immune response in ovariectomized female mice during blocking of endogenous PG synthesis. 1) Injection of solvent of hormone; 2) injection of solvent of hormone and PG-synthetase inhibitor; 3) injection of CG (40 U) and of PG-synthetase inhibitor; 4) injection of CG (200 U) and of PG-synthetase inhibitor.

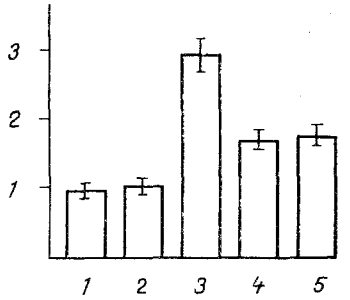

Fig. 3. Effect of CG on IL-2 production by splenocytes of female mice. Ordinate, index of stimulation of DNA synthesis. 1) Thymocytes; 2) thymocytes + mitogen; 3) thymocytes + mitogen + IL-2 from supernatants of control cultures; 4) thymocytes + mitogen + IL-2 from supernatants of experimental cultures (CG — 20 U/ml); 5) thymocytes + mitogen + IL-2 from supernatants of experimental cultures (CG — 100 U/ml).

not only at the cellular, but also at the molecular level. PG and IL-2 are known to play an important role in the regulation of cooperation between lymphoid cells and macrophages, and to possess reciprocal properties relative to one another [5]. An attempt was made to evaluate the role of these mediators in the immunomodulating action of CG.

Addition of a PG-synthetase inhibitor to the functional system (Figs. 1 and 2) significantly inhibited the formation of a primary immune response in both ovariectomized and intact animals, confirming the important role of endogenous PG in antigen-dependent differentiation of immunocompetent cells [14]. Injection of GC (40 or 200 U) into the animals against the background of blockade of synthesis of endogenous PG by the residual gonads abolished the inhibitory effect of the PG-synthetase inhibitor. The impression was obtained that sex steroids realized their immunostimulating action independently of the PG system, than which they

are no less effective, for under these experimental conditions an immune response is formed at a level equal to the control. In ovariectomized animals in which synthesis of endogenous PG was blocked, the immunodepressive effect of the hormone completely disappeared. This finding suggests that the mechanism of the immunomodulating effects of CG operates through the PG system.

In the experimental model in vitro an inhibitory action of various doses of CG on mitogenic induction of IL-2 by the splenocytes was observed (Fig. 3), directly controlling proliferation and differentiation of B lymphocytes at the antigen-dependent stage [7]. The quantity of IL-2 in the supernatant of the experimental cultures was independent of the dose of CG, evidence that competitive relations do not exist between CG and the mitogen. The hormone may either have a direct inhibitory effect on the IL-2 producing cells or may inhibit synthesis of IL-2 mediated through PG. Experiments in a syngeneic transfer system suggests that the second hypothesis is more likely.

It can thus be postulated that CG inhibits the formation of the primary immune response by disturbing cooperative relations of the immunocompetent cells at the level of short-distance mediators of immunity. Under these circumstances the hormone raises the level of the immunologically important class of PG above its optimal value, as a result of which the functional activity of the helper T cells, secreting IL-2, is depressed.

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