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Effect of Chorionic Gonadotropin on the Formation of the Secondary Immune Response

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Chorionic gonadotropin (CG) synthesized by human placenta, controls hormone-dependent mechanisms of the growth and development of the fetoplacental complex from the moment of ovum implantation to birth [1]. A semiallogenic fetus and placenta are recognized by maternal lymphocytes as foreign antigenic material but are not rejected by them for a number of reasons [4]. An important role is played here by pregnancy hormones which are potent immunosuppressants [5].

CG is a highly active modulator of immune reactions. A dose-dependent suppression of the cellular [7,11] and humoral [2,9] immune response by the hormone has already been demonstrated, but as a rule only with respect to the effect of CG on the primary immune response of immunocompetent cells. Relationships between the maternal immunity system and the fetoplacental complex include not only the primary, but also a secondary, or anamnestic, immune response, which becomes dominant in repeated pregnancies [3].

We have investigated the immunomodulatory effects of CG and ovarian sex steroids as its possible mediators on the genesis of the anamnestic immune response.

MATERIALS AND METHODS

Experiments were carried out with female CBA mice weighing 18 to 22 g. One group of animals was subjected to bilateral ovariectomy under ether anesthesia. These animals were used in experiments one month after the operation.

The scheme of secondary immune response induction consisted of two stages. At stage I the animals were intraperitoneally immunized with sheep erythrocytes (SE) in a dose of 2×10^8 cells and 20 days later (stage II) were again immunized with the same dose of SE. The secondary immune response was assessed on day 5 after reimmunization by local hemolysis in agarose gel [8]. Antibody (IgG) producing cells were determined by a

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modification of the indirect Jerne method [6] using pretitered rabbit antiserum to murine IgG (Diagnostikum) in dilution 1:200.

CG (Profasi, Serano, Italy) was subcutaneously injected the day following the repeated immunization and once more on day 3 after the repeated immunization, that is on days 1 and 3 of anamnestic immune response formation. The hormone was used in two doses which were extrapolated from the mean serum concentrations of CG in pregnant women during trimesters I and II-III and were 200 and 40 IU, respectively [12]. To rule out immunomodulatory effects of CG mediated by the ovarian sex steroids, we used not only oophorectomized female mice, but also direct *in vitro* exposure of splenocytes to the hormone. For this purpose only a primary immune response was induced in intact females; 20 days later they were sacrificed, and splenocytes were isolated under aseptic conditions and incubated for one hour at 37°C in medium 199 with CG in a dose of 40 or 200 IU per culture. A macroculture variant was used: 2×10^7 nucleated splenocytes in 4 ml of medium 199. Splenocytes incubated under similar conditions without hormone served as controls. After a one-hour incubation the splenocytes were washed three times in medium 199 and concentrated to attain a concentration of 2×10^7 nucleated splenocytes per 0.5 ml medium. This suspension, together with SE (2×10^8), was then injected into the caudal vein of lethally irradiated (219.3 mCi/kg) syngeneic recipient mice. The adoptive secondary immune response was assessed as described above. Splenocyte viability after culturing, assessed with trypan blue, was 92 to 98%. Thus, not only were immunomodulatory effects mediated by sex steroids ruled out *in vitro*, but the effect of the hormone on repeatedly primed cells as well.

The results were statistically processed using Student's *t* test with counts of antibody-producing cells used in all estimations.

RESULTS

Administration of CG to intact female mice had no statistically significant effect on the levels of IgM- and IgG-producing cells. On the other hand, injections of high (200 IU) hormone doses markedly suppressed the number of nucleated splenocytes in intact mice (Table 1). The ratio of IgM- and IgG-producing cells was unchanged: 1 to 10.

Oophorectomy did not affect the counts of IgM- and IgG-producing cells determining the secondary immune response, nor did it have an effect on splenocyte counts. Injection of CG in a dose of 40 IU to oophorectomized animals did not alter the processes of anamnestic immune response formation (Table 1). Comparison of the results of CG injection in a dose of 40 IU to oophorectomized and intact animals did not reveal a reliable difference between the responses. The hormone in a dose corresponding to its concentrations during the second and third trimesters of pregnancy did not appear to mediate its immunomodulatory effects through the ovarian sex steroids, as was previously observed during the primary immune response [2]. When injected to oophorectomized mice in a dose of 200 IU the hormone reliably inhibited the secondary immune response and splenocyte counts. Note that the suppression of antibody-producing cells is

TABLE 1. Effect of Chorionic Gonadotropin on Secondary Immune Response in Intact and Oophorectomized Animals (Mean±SEM)

Group	Experimental agent	Nucleated splenocytes, $\times10^6$	log IgM-producing cells in spleen	log IgG-producing cells in spleen
1 (n=11)	Control	232.7±15.8	3.703±0.084 (6138.0)	4.683±0.90 (58064.1)
2 (n=12)	CG (40 IU)	192.9±15.3	3.730±0.100 (6926.5)	4.758±0.071 (64376.0)
3 (n=10)	CG (200 IU)	183.0±11.1 $p_{3-1}<0.02$	3.591±0.059 (4263.8)	4.652±0.095 (53472.0)
4 (n=9)	Control, oophorectomy	224.3±9.8	3.675±0.052 (5029.4)	4.802±0.070 (69438.9)
5 (n=11)	Oophorectomy, CG (40 IU)	225.1±16.0	3.649±0.063 (5029.8)	4.822±0.072 (76188.1)
6 (n=12)	Oophorectomy, CG (200 IU)	156.0±6.8 $p_{6-4}<0.001$ $p_{6-5}<0.001$	3.536±0.044 (3651.5)	4.601±0.038 (41691.2)

Note. Here and in Table 2 control animals were injected with sterile normal saline (hormone solvent); n = number of animals. Only reliable values in comparison of relevant group pairs are presented. In parentheses: absolute counts of antibody-producing cells.

TABLE 2. Effect of Chorionic Gonadotropin *in Vitro* on Splenocyte Capacity to Form Adoptive Secondary Immune Response (Mean±SEM)

Group	Experimental agent	log IgM-producing cells (2×10^7)	log IgG-producing cells (2×10^7)
1 ($n=21$)	Control	3.057±0.051 (1301.5)	3.078±0.040 (1301.8)
2 ($n=12$)	CG (40 IU/4 ml)	3.019±0.104 (1399.8)	3.118±0.072 (1513.8)
3 ($n=11$)	CG (200 IU/4 ml)	2.814±0.073 (737.6)	2.917±0.082 (960.3)

selective, relating only to IgG-producers; even in a high dose CG had no effect on IgM-producing cells (Table 1). The statistically reliable difference between counts of IgG-producers in oophorectomized animals after CG injections in doses of 40 and 200 IU is indicative of a dose-dependent immunosuppressive effect of the hormone.

Hence, we may speak of an independent immunosuppressive effect of high doses of CG, which manifests itself in suppression of IgG-producing cells, the principal antibody producers in the secondary immune response. It is possible that this effect of the hormone is explained by its ability to reduce splenocyte count. The ability of ovarian sex steroids to level the depressive effect of high-dose CG on the formation of IgG-producing cells is to be noted.

Addition of the hormone to a splenocyte culture in a dose of 40 IU had no noticeable effect on the counts of IgM- or IgG-producing cells forming in the body of irradiated recipients, but when added in a dose of 200 IU it reliably inhibited splenocyte capacity to form an anamnestic immune response. In contrast to *in vivo* experiments, *in vitro* CG suppressed IgM-producing cells (Table 2). This appears to be related to the level of activation of antibody-producer precursors. While CG was injected to oophorectomized animals after reimmunization, *in vitro* it interacted with the cells sensitized but not repeatedly activated by the antigen. We may conclude from this that CG-dependent immunodepression is associated not only with the hormone dose, but also with the moment of exposure to antigen, which appears to select IgM- or IgG-producing cells for the regulatory effect of CG.

Hence, chorionic gonadotropin in a dose typical of weeks 7-10 of pregnancy is capable of suppressing the secondary immune response by lowering the counts of IgM- or IgG-antibody producers. The selective type of the hormone effect is due to the level and number of antigenic activations of the pool of antibody producer precursors. Sex steroids, whose concentration increases for exposure to CG [1], level the immunosuppressive effect of this pregnancy hormone but cannot eliminate its suppressive effect on the splenocyte count. From the viewpoint of pregnancy physiology, the ability of chorionic gonadotropin to suppress the formation of the secondary immune response only when used in a high dose characteristic for its peak secretion is biologically justified, because it is during this period that the principal histocompatibility antigens are expressed on fetal cells [10].

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