# EFFECT OF CHORIONIC GONADOTROPHIN ON DYNAMICS OF THE PRIMARY IMMUNE RESPONSE

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The phenomenon of hormonal regulation of the immune system is being vigorously studied, but investigations of the immunomodulating properties of individual hormones have been undertaken mainly at optimal times of the immune response. Most hormones can influence metabolism in lymphoid tissue [2, 3], which may lead to a shift in the time frames of immune reactions. When studying processes of endocrine control of immunogenesis, it is therefore important to know the dynamics of development of the immune response.

For pregnancy to run a successful course, regulation of the primary immune response is very important, for in the course of its formation and development several tasks connected with the formation of memory cells and switching from synthesis of antibodies from the M to the G class, particular isotypes of which can behave as blockers of maternal effector cells, and thus save the semiallogeneic fetus from destruction, have to be successfully performed.

The principal hormone of reproduction, chorionic gonadotrophin (CG) can modulate the formation of antibody-forming cells (AFC) [1, 6], although the action of the hormone on switching of antibody synthesis in the course of the primary immune response has not yet been established.

The aim of this investigation was to study whether CG can affect the dynamics of the primary immune response and the processes of switching of antibody synthesis linked with it.

## EXPERIMENTAL METHOD

Female CBA mice weighing 20-22 g were used. Ovariectomy was performed on some of the animals under ether anesthesia. The immune response was induced by intraperitonesl injection of sheep's red blood cells in a dose of $2 \cdot 10^8$ cells. The AFC level was estimated by the local hemolysis in agarose gel reaction [5] on the 5th, 8th, and 12th days after immunization, Starting with the 8th day, besides direct (IgM) AFC, the indirect (IgG) AFC also were determined, by the method in [4]. A standard rabbit antiserum against mouse IgG (Gamaleya Institute of Epidemiology and Microbiology, Moscow) was used in a titer of 1:200, which was established empirically. The hormone ("Profasi," Italy) was injected into both intact and ovariectomized animals on the day after immunization, and thereafter on alternate days in doses of 200 or 40 IU, corresponding to the physiological CG concentration in the I and II-III trimesters respectively. Thus animals whose immune response was assessed on the 5th day received a total of two injections, on the 8th day – four injections, and on the 12th day – six injections.

The experimental results were subjected to statistical analysis by Student's t test. In all series, the operative parameter was $\log_{10}$ of the number of AFC.

## EXPERIMENTAL RESULTS

Injection of CG in a dose of 40 IU into nonovariectomized animals led to a statistically significant increase in the number of M-AFC on the 5th day of the primary immune response. A dose of 200 IU had no significant effect. In ovariectomized animals neither dose of the hormone exhibited an immunomodulating action (Table 1).

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TABLE 1. Effect of CG on IgM-AFC Formation in Spleen of Intact and Ovariectomized CBA Mice by 5th Day of Primary Immune Response (M ± m, n = 8-10)

| Group No. | Experimental conditions | NC in spleen, $\times 10^6$ | IgM-AFC per organ |
|---|---|---|---|
| 1 | Control (injection of solvent of CG) | 227,2±31,6 | 4,565±0,115(47332,5) |
| 2 | Injection of CG (40 IU) | 239,0±24,0<br>$p_{2-1}>0,05$ | 4,926±0,062(90457,5)<br>$p_{2-1}<0,02$ |
| 3 | Injection of CG (200 IU) | 214,7±34,2<br>$p_{3-1}>0,05$ | 4,614±0,102 (51623,0)<br>$p_{3-1}>0,05$ |
| 4 | Ovariectomy, control | 228,6±20,4 | 5,143±0,054 (148333,6) |
| 5 | Ovariectomy, injection of CG (40 IU) | 198,7±11,4<br>$p_{5-4}>0,05$ | 5,220±0,023 (168513,3)<br>$p_{5-4}>0,05$ |
| 6 | Ovariectomy, injection of CG (200 IU) | 187,2±15,2<br>$p_{6-4}>0,05$ | 5,220±0,049 (175781,0)<br>$p_{6-4}>0,05$ |

**Legend.** Here and in Tables 2 and 3: numbers in parentheses are mean values in natural numbers; NC – nucleated cells.

TABLE 2. Effect of CG on Formation of IgM- and IgG-AFC in Spleen of Intact and Ovariectomized CBA Mice by 8th Day of Primary Immune Response (M ± m, n = 10-11)

| Group No. | Experimental conditions | NC in spleen, $\times 10^6$ | $I_gM$-AFC per organ | IgG-AFC per organ |
|---|---|---|---|---|
| 1 | Control | 223,1±13,4 | 3,991±0,103<br>(12 474,5) | 4,241±0,062<br>(19 315,8) |
| 2 | Injection of CG (40 IU) | 225,9±26,5<br>$p_{2-1}>0,05$ | 3,831±0,112<br>(8 717,7)<br>$p_{2-1}>0,05$ | 3,866±0,170<br>(13 717,5)<br>$p_{2-1}>0,05$ |
| 3 | Injection of CG (200 IU) | 209,0±16,3<br>$p_{3-1}>0,05$ | 3,641±0,075<br>(5073,4)<br>$p_{3-1}<0,02$ | 3,924±0,083<br>(9 840,4)<br>$p_{3-1}<0,01$ |
| 4 | Ovariectomy, control | 196,4±13,5 | 4,321±0,066<br>(23 394,7) | 4,734±0,060<br>(59 104,5) |
| 5 | Ovariectomy, injection of CG (40 IU) | 158,6±7,1<br>$p_{5-4}<0,05$ | 4,395±0,056<br>(27 053,4)<br>$p_{5-4}>0,05$ | 4,458±0,064<br>(32 458,2)<br>$p_{5-4}<0,01$ |
| 6 | Ovariectomy, injection of CG (200 IU) | 156,4±3,9<br>$p_{6-4}<0,02$ | 4,331±0,063<br>(23 473,4)<br>$p_{6-4}>0,05$ | 4,659±0,064<br>(50 267,2)<br>$p_{6-4}>0,05$ |

On the 8th day of the primary immune response M- and G-AFC were formed in the intact mice under the influence of CG (40 IU), on a level comparable with the control, whereas injection of the hormone in a dose of 200 IU led to a significant reduction in the number of M- and G-AFC. After ovariectomy, CG in a dose of 40 IU reduced the number of nucleated cells (NC) in the spleen and selectively inhibited the number of G-AFC without affecting the level of IgM-AFC. In a dose of 200 IU the hormone had no significant effect on either M- or G-AFC, but significantly reduced the number of NC in the spleen (Table 2).

On the 12th day of the primary immune response levels of M- and G-AFC in intact mice receiving CG in a dose of 40 IU were unchanged but the number of cells in the spleen was reduced. By contrast, in a dose of 200 IU, whereas the hormone did not affect the NC level, it inhibited M- and G-AFC. After ovariectomy, CG exhibited an immunodepressive action on G-AFC formation only in a high dose, without affecting the level of M-AFC. The number of NC in the spleen under these circumstances was unchanged (Table 3).

The results show that at the antigen-dependent stage of splenocyte differentiation into AFC, CG exhibits immunomodulating effects through ovarian hormones; the dose of CG, moreover, plays an important role, for only if a high dose of the hormone is given is the formation of both M-and G-AFC inhibited. Suppression of M-AFC by CG on the 8th and 12th days only in nonovariectomized animals is evidence of synergism of the immunosuppressive effects of CG and sex steroids. Ovarian hormones, induced by the gonadotrophin can evidently inhibit only M-AFC. So far as the effects of CG itself are concerned, two factors can be distinguished in this case: 1) the hormone does

TABLE 3. Effect of CG on IgM- and IgG-AFC Formation in Spleen of Intact and Ovariectomized CBA Mice by 12th Day of Primary Immune Response (M ± m, n = 10-11)

| Group No. | Experimental conditions | NC in spleen, $\times 10^6$ | IgM-AFC per organ | IgG-AFC per organ |
|---|---|---|---|---|
| 1 | Control | 221,5±10,0 | 3,402±0,057 (2743,2) | 4,358±0,069 (25 697,5) |
| 2 | Injection of CG (40 IU) | 169,1±7,1 $p_{2-1}<0,001$ | 3,305±0,047 (2139,8) $p_{2-1}>0,05$ | 4,253±0,098 (21 573,8) $p_{2-1}>0,05$ |
| 3 | Injection of CG (200 IU) | 206,4±11,0 $p_{3-1}>0,05$ | 3,127±0,070 (1503,5) $p_{3-1}<0,01$ | 3,854±0,128 (10 791,6) $p_{3-1}<0,01$ |
| 4 | Ovariectomy, control | 178,7±12,9 | 3,290±0,040 (2040,0) | 4,233±0,037 (17 750,1) |
| 5 | Ovariectomy, injection of CG (40 IU) | 206,3±9,6 $p_{5-4}>0,05$ | 3,412±0,078 (2934,9) $p_{5-4}>0,05$ | 4,084±0,062 (13 358,3) $p_{5-4}>0,05$ |
| 6 | Ovariectomy, injection of CG (200 IU) | 171,3±10,8 $p_{6-4}>0,05$ | 3,223±0,061 (1835,4) $p_{6-4}>0,05$ | 3,857±0,038 (7 486,8) $p_{6-4}<0,001$ |

not affect the M-AFC level in the course of the primary immune response, regardless of its dose, but it selectively inhibits IgG-producing cells, and thereby regulates the switching of antibody synthesis; 2) selective depression of G-AFC depends on the dose of CG and on the time course of the process. In a small dose the effect is realized at the early stages of switching (the 8th day), but in a large dose, at late stages (the 12th day). Moreover, whereas for a dose of 40 IU this effect can be explained by its ability to reduce the number of NC in the spleen by the 8th day and to restore their number by the 12th day, it is impossible to explain immunodepression by a dose of 200 IU through regulation of the cell content of the organ, for the curves reflecting the time course of the number of NC in the spleen and the immunodepressive activity of this dose intersect.

Injection of CG into intact animals in a dose reflecting its level in the I trimester of pregnancy (200 IU) thus significantly alters the dynamics of the primary immune response. The results obtained on ovariectomized animals lead to the conclusion that immunodepression can be effected both by CG itself, which selectively inhibits G-AFC, and by ovarian sex steroids induced by it, and which evidently respond to abortive termination of the primary immune response in the course of time, without disturbing its realization at the peak of M-AFC production. It will be noted that in a dose characteristic of the II-III trimesters of pregnancy (40 IU) despite its ability to suppress the switching of immunoglobulin synthesis as early as on the 8th day of the immune response, CG does not exhibit this activity in animals with intact ovaries, but intensifies the release of M-AFC at the optimal times of their formation.

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