Sensitivity of Immunocompetent Cells to a Hormonal Signal during Mitogenic Exposure

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An understanding of the processes of immune system endocrine regulation is closely connected with studies of the mechanisms determining lymphoid cell sensitivity to a hormonal signal. Mammalian immunocompetent cells are capable of interacting with various hormones due to the expression of specific receptors on their surface [8-10]. Investigation of the immunomodulatory effects of some hormones revealed a clear-cut correlation between the intensity and direction of their effects and the time and type of antigenig effect [3,5]. The same cells reacting to alloantigens in a mixed lymphocyte culture were found to express receptors to various hormones during different phases of the reaction [1]. Studies of the immunomodulatory influences of reproductive hormones have shown variously directed effects of chorionic gonadotropin (CG) on the processes of antibody-producing cells (APC) formation during various stages of immunocyte differentiation and depending on their appurtenance to the T and B series [7].

The present research was aimed at elucidating the relationship between the immunomodulatory effects of chorionic gonadotropin and the functional activity of lymphoid splenocytes.

MATERIALS AND METHODS

CBA and (CBA×C57B1/6)F_1 male mice weighing 18-22 g were used in the experiments. Splenocytes of intact animals were incubated at 37°C for one hour as a macroculture: 2×10^7 cells in 4 ml of serum-free medium 199. Cell viability was 95% on average. The cells were then washed in medium 199 and their concentration was brought to 2×10^7 in 0.5 ml; a cell suspension with sheep erythrocytes (2×10^8) was injected into the caudal vein of lethally irradiated (219.3 mCi/kg) syngeneic recipients. The adoptive immune response was assessed on day 4-5 after syngeneic transfer by local hemolysis in agarose gel after Jerne [12]. Chorionic gonadotropin (Prafasi, Serano, Italy) (CG) (0.2 ml) was added to each experimental splenocyte macroculture in doses corresponding to its concentrations in the first and second-third trimesters of pregnancy, 200 and 40 IU, respectively [15]. In some experiments B lymphocyte mitogens were added to the splenocyte culture in addition to CG: lipopolysaccharide (LPS) in a dose of 50 μg/ml (Diagnosticum) or murine anti-IgM monoclonal antibodies (Calbiochem, USA) in a dose of 10 μg/ml. In some experiments with LPS macrophages were pre-eliminated from the splenocyts by active adhesion on the glass [2]. Cell cultures with nothing but medium 199 served as control.

The results were processed using the Student t test. All operations were carried out with the log of the APC counts.

RESULTS

The one-hour incubation of intact splenocytes with CG in doses of 40 or 200 IU resulted in a statistically reliable reduction of the APC count. The addition of LPS to the nonfractionated splenocyte culture

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TABLE 1. Effect of Chorionic Gonadotropin on Capacity of Intact and LPS–Activated Splenocytes to Form an Active Immune Response ($M \pm m$)

Group №.	Experimental exposure	Log of APC number per 2×10^7 cells
1 ($n=40$)	Control	3.237±0.033 (1926.5)
2 ($n=8$)	CG (40 IU)	2.893±0.103 (947.5) $p_{2-1}<0.002$
3 ($n=12$)	CG (200 IU)	2.949±0.047 (950.0) $p_{3-1}<0.001$, $p_{3-2}>0.05$
4 ($n=11$)	LPS (50 μg/ml)	3.284±0.076 (2227.2) $p_{4-1}>0.05$
5 ($n=12$)	LPS + CG (40 IU)	3.668±0.052 (5068.3) $p_{5-1}<0.001$, $p_{5-4}<0.001$, $p_{5-2}<0.001$
6 ($n=8$)	LPS + CG (200 IU)	3.551±0.051 (3755.0) $p_{6-1}<0.001$, $p_{6-4}<0.01$, $p_{6-3}<0.001$, $p_{6-5}>0.05$

Note. n – number of animals per group; in control – absolute APC count.

failed to noticeably change the level of the adoptive immune response, whereas its addition together with CG had a marked immunostimulating effect (Table 1).

LPS is known not only as a B lymphocyte polyclonal activator, but as a potent inductor of interleukin-1 and tumor necrosis α-factor synthesis by macrophages [14]. These cytokines determine to a considerable extent the processes of B lymphocyte differentiation into APC precursors, and therefore negative macrophage selection was carried out to rule out cytokine effect. Table 2 shows that splenocytes free of macrophages reduce several-fold APC formation in response to thymus-dependent antigen, this indicating the efficacy of the selection made. The addition of a high hormonal dose to such a cell culture did not influence the level of the adoptive immune response. Splenocytes in the absence of macrophages appeared to be no longer sensitive to the depressive effect of CG. At the same time, CG in a low dose exerted an opposite, immunostimulating effect. Previous *in vivo* experiments have demonstrated that the immunostimulating effect of CG (in a dose of 40 IU) is connected with a selective activation of B lymphocytes [6]. Hence, one may speak of a selective immunostimulating effect of a low dose of CG as a direct function of the presence of macrophages.

One-hour LPS incubation with splenic lymphocytes in the absence of macrophages resulted in a twofold increase of APC outflow to thymus antigen (Table 2). The presence of CG together with LPS levels the effect of B lymphocyte polyclonal activator. To specify the mechanisms of hormonal modulation of activated splenic B lymphocytes a highly specific B lymphocyte activator was used, monoclonal anti-IgM antibodies transferring cells into B blasts in the same way as LPS [13], but not involving the macrophagal and T lymphocyte cytokine cascade. It is shown in Table 3 that the addition of anti-IgM antibodies to the nonfractionated splenocyte culture enhanced APC formation in the adoptive immune response. The addition of CG (40 or 200 IU) in parallel with the addition of anti-IgM antibodies had no modulating effect on the immunostimulating action of the antibodies. In other words, immunomodulatory effects of CG are not observed in the presence of selective blocking of membrane IgM receptors leading to B lymphocyte activation.

Summing up the results, we would note the following:

First, CG in doses compatible with the hormone concentrations during normal pregnancy is character-

TABLE 2. Effect of Chorionic Gonadotropin on Capacity of Intact and LPS–Activated Splenocytes Devoid of Adhesive Cells to Form Adoptive Immune Response ($M \pm m$)

Group №.	Experimental exposure	Log of APC number per 2×10^7 cells
1 ($n=44$)	Control	2.618±0.030 (462.7)
2 ($n=16$)	CG (40 IU)	2.769±0.052 (655.0) $p_{2-1}<0.01$
3 ($n=13$)	CG (200 IU)	2.570±0.060 (415.3) $p_{3-1}>0.05$, $p_{3-2}<0.02$
4 ($n=8$)	LPS (50 μg/ml)	3.016±0.070 (1135.0) $p_{4-1}<0.001$
5 ($n=10$)	LPS + CG (40 IU)	2.697±0.075 (576.0) $p_{5-1}>0.05$, $p_{5-4}<0.01$, $p_{5-2}>0.05$
6 ($n=10$)	LPS + CG (200 IU)	2.590±0.072 (442.0) $p_{6-1}>0.05$, $p_{6-4}<0.001$, $p_{6-3}>0.05$, $p_{6-5}>0.05$

TABLE 3. Effect of Chorionic Gonadotropin on Capacity of Splenocytes Activated with Anti-IgM Antibodies to Form Adoptive Immune Response ($M \pm m$)

Group №.	Experimental exposure	Log of APC number per 2×10^7 cells
1 ($n=18$)	Control	3.128±0.045 (1468.8)
2 ($n=10$)	Anti-IgM antibodies (10 μg/ml)	3.413±0.085 (3186.0) $p_{2-1}<0.01$
3 ($n=10$)	Anti-IgM antibodies+CG (40 IU)	3.439±0.061 (2990.0) $p_{3-1}<0.001$, $p_{3-2}>0.05$
4 ($n=4$)	Anti-IgM antibodies+CG (200 IU)	3.485±0.055 (3135.0) $p_{4-1}<0.001$, $p_{4-2}>0.05$, $p_{4-3}>0.05$

ized by a marked immunosuppressive effect realized by cells adhering to glass, provided that the lymphoid cells are at the stages of antigen-independent differentiation, that is, nonactivated.

Second, macrophage removal abolished the immunosuppressive effect of a high dose of CG but permitted the hormone to show its opposite, immunostimulating effect at a low dose, which is possible only on the condition of antigen-independent B lymphocyte differentiation. It seems that the macrophage-mediated suppression either blocks B lymphocyte activation with a low CG dose or that macrophages bind the hormonal molecules competing for them and thus leave the B lymphocytes without a triggering signal. The former hypothesis seems to be more plausible, as otherwise a high CG dose could not have a suppressive effect.

Third, during splenocyte activation with LPS the hormone acts a co-stimulating agent enchancing the mitogen effect.

Macrophages again become target cells for this effect, and their removal from the suspension eliminates the immunostimulating effect of CG. The complete absence of CG immunomodulating effects in the presence of anti-IgM antibodies in reason to think that the hormone selectively stimulating B lymphocytes triggers transduction mechanisms similar to anti-IgM antibodies. LPS and anti-IgM antibodies are known to trigger B lymphocyte activation processes by increasing the concentration of intracellular Ca^{2+}, but whereas LPS does so only at the expense of extracellular Ca^{2+}, antibodies do so exlusively due to the release of the intracellular Ca^{2+} reserves [11]. The hormonal depression of LPS-activated B lymphocytes seems to be related to the simultaneous switching on of both channels of Ca^{2+} entry into cells, this resulting in a surplus and, as a rule, the formation of insoluble Ca^{2+} phosphate salts, thereby blocking energy processes in the cell [4].

Hence, the effects of CG depend entirely on the composition and functional activity of immunocompetent cells. It is thus possible to speak of the endocrine regulation of immunogenetic processes, but the problem of the hierarchical relationships between the immune and endocrine systems has yet to be solved. The hormone acts as a factor whose regulating signal will be adopted by the immune system if it is needed. This, in its turn, determines the direction of hormonal signal realization: it will be the direction needed by the cell receiving the hormone.

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