about serum-induced disorders in normal ion ditribution. Another ion-transporting system of the cell, consisting of chemo- and potential-regulated channels, may be disturbed as well. Ions cross the membrane via these ion-selective channels along their concentration gradient. The state of ion channels is known to be regulated by many factors, among which are some substances acting on the external surface of the membrane.

The above facts suggest that the de- and hyperpolarization changes detected in the first few minutes may reflect the effects of substances in the serum on regulatory subunits of the ion channel. Intravenous laser therapy in the majority of cases reduces the activity of these factors present in patients' sera which probably disturb the normal functioning of the ion-transporting systems of the neuronal membrane.

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The Costimulating Effect of Chorionic Gonadotropin on Lymphokine-Activated Splenocytes. New Aspects of the Immunomodulating Effect of the Pregnancy Hormone

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Chorionic gonadotropin (CG) is known to possess a well expressed immunomodulating activity. As a rule, the effects of the hormone are of a dose-dependent nature and can change direction depending on the differentiation level of immunocytes and the presence of female steroid hormones [1,3,4]. Since CG is a pregnancy hormone, investigation of its immunomodulating effects is of great importance for understanding the mechanisms of immunological tolerance formation in the maternal organism vis-a-vis the semiallogenic fetus. The CG secretion by neoplastic cells [10] and the hormone's ability to intensify carcinogenesis and metastasis [6] give grounds for regarding this hormone as a factor promoting development of the neoplastic process, which always goes along with immune mechanism disorders.

The object of the present study was to examine the effect of CG on the processes of antigen-independent differentiation of immunocompetent cells under the influence of differentiative signals varying in level and quality.

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MATERIALS AND METHODS

The experiments were performed on female CBA mice weighing 20-22 g. Chorionic gonadotropin

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(Profasi, Italy) in a dose of 40 or 200 IU was added to a short-term macroculture of splenocytes (2×10^7 cells in 4 ml of medium 199) and incubated at 37°C for 60 min. The cells were washed to remove the hormone and concentrated to a density of 2×10^7 cells in 0.5 ml medium. Subsequently, the cells were intravenously transferred together with the antigen (sheep erythrocytes, 2×10^8/0.2 ml) to syngeneic, lethally irradiated (219.3 mCi/kg) recipients. The control cells were subjected to the same manipulations without the hormone.

The effect was assessed from the adaptive immune response level, determined on the 4th-5th day after syngeneic transfer by the method of local hemolysis in agarose gel [8]. Splenocytes were activated by adding to the cell culture either concanavalin A (ConA) or interleukin-2 (IL-2) together with or separately from CG. Since T lymphocytes are the main target cells for these agents, the CG effects were interpreted for this lymphocyte subpopulation. IL-2 (recombinant human, Diagnostikum, Russia) was used in a dose of 150 IU/4 ml; ConA (Calbiochem) was added to a final concentration of 5 μg/ml or 20 μg/4 ml culture.

Cell viability assessed in the test with the vital stain trypan blue constituted 94-98%.

The results were subjected to statistical analysis using Student's t test. For all calculations $\log_{10}$ (log) of the number of plaque-forming cells (PFC) was determined.

RESULTS

Short-term incubation of splenocytes with CG in doses corresponding to its blood concentration during the first (200 IU) and second-third (40 IU) trimesters of pregnancy [9] results in a statistically significant suppression of the adaptive immune response (Table 1). This testifies to the hormone's ability to inhibit the processes of antigen-independent lymphocyte differentiation into PFC precursors (PFCP). As established by us earlier, CG in physiological doses suppresses ConA-induced synthesis of IL-2 by splenocytes of female mice [3]

TABLE 1. Effect of Chorionic Gonadotropin on Formation of Plaque-Forming Cells by Intact Splenocytes in Syngeneic Transfer ($M\pm m$)

Experimental conditions	log PFC/2×10^7 cells
Control ($n=40$)	3.237±0.033 (1926.5)
CG, 40 IU ($n=8$)	2.893±0.103 (947.5)
CG, 200 IU ($n=12$)	2.949±0.047 (950.0)

Note. Here and in Tables 2 and 3 the absolute number of PFC is indicated in parentheses.

TABLE 2. Effect of Chorionic Gonadotropin and ConA on PFC Formation by Intact Splenocytes in Syngeneic Transfer ($M\pm m$)

Experimental conditions	log PFC/2×10^7 cells
Control ($n=18$)	3.261±0.062 (2177.7)
ConA, 20 μg ($n=10$)	3.380±0.101 (2946.0)
ConA + CG, 40 IU ($n=10$)	3.205±0.048 (1694.0)
ConA + CG, 200 IU ($n=10$)	3.077±0.081 (1416.0)

without affecting the functional activity of helper T lymphocytes of the humoral immune response, provided that there has been no cell activation by ConA [4].

As follows from Table 2, addition of ConA in a concentration inducing endogenous IL-2 production does not evoke any significant rise in PFC content. Nor does the simultaneous presence of CG (40 IU) and ConA influence the splenocytes' capacity for PFC formation in the syngeneic transfer. However, in a higher dose (200 IU) the hormone provokes a reduction of the PFC number. It is probable that different CG doses realize their immunomodulating effect via different lymphocyte populations, their activation by lectins counteracting the suppressive effect of a low dose of hormone.

A one-hour incubation of splenocytes with exogenous IL-2 does not affect the processes of PFCP formation, a fact which confirms once again the multicomponent character of cell-cell interactions at the stage of antigen-independent differentiation of immunocompetent cells realizing the primary immune response. The joint presence of IL-2 and CG (200 IU) in the splenocyte culture leads to a pronounced effect, manifested in a more than 2-fold increase of PFC formation. On the other hand, CG in a dose of 40 IU in an analogous experiment does not produce any significant effect (Table 3).

Thus, a costimulating effect of the high CG dose on the processes of antigen-independent splenocyte differentiation into PFCP has been revealed. As is known, T lymphocytes bear receptors to IL-2 [7]. When added separately, recombinant IL-2 and CG either do not affect or even inhibit the processes of formation of PFC to thymus-dependent antigens. In our experiments, stimulation of these processes for the joint influence of recombinant IL-2 and CG may be a result of the syngeneic effect of the hormone and lymphokine on T helpers. As for the phenomenology of the costimulating effect of the high CG dose, in our opinion, we are dealing here with some hitherto unknown selective immunotrophic effect of the pregnancy hormone on T helpers. This effect consists either in the activation of exogenous IL-2

TABLE 3. Effect of CG and IL-2 on PFC Formation by Intact Splenocytes in Syngeneic Transfer ($M \pm m$)

Experimental conditions	log PFC/2×10^7 cells
Control ($n=40$)	3.237±0.033 (1926.50)
IL-2, 150 IU ($n=9$)	3.282±0.095 (2253.3)
IL-2 + CG, 40 IU ($n=9$)	3.299±0.110 (2600.0)
IL-2 + CG, 200 IU ($n=10$)	3.587±0.071 (4344.0)

receptor expression, leading to a significant increase in the pool of activated helpers, or in that CG acts as a B lymphocyte-activating signal which, together with IL-2, promotes differentiation of B lymphocytes into PFCP. Since in a dose of 200 IU CG suppresses the ConA-activated lymphocytes and produces a costimulating effect, we believe that the selective effect of CG on helper T lymphocytes is the most likely supposition. If this is the case, the direction of the immunomodulating effect of the high dose of hormone is determined by the quality of the T lymphocyte-activating signal.

As has been recently established, maternal lymphocytes act not only as potential aggressors of the placenta and fetus but as trophic agents as well. Cytokines and lymphokines secreted by lymphocytes in the trophoblast zone facilitate the development of a normal pregnancy and a live birth [5]. From this standpoint, the costimulating effect of the high dose of CG may be interpreted as a phenomenon of a universal character. It is most important in the case of trophoblast cells, since in this zone the hormone concentration exceeds several times that in the peripheral blood of the maternal organism.

If ConA is regarded as an antigenic stimulus for T lymphocytes, then IL-2 acts as a secondary signal controlling the processes of amplification and expansion of the already committed T-cell clone [2]. In view of this, CG may be regarded as a feto-placental protector of biphasic action. On the one hand, its major effect is directed toward blocking the afferent component in the humoral immune response, while on the other, this protector is capable of intensifying the efferent component of the immune response. The strict dose dependence of the CG costimulating effect testifies to a limited possibility of realizing this effect during the physiological course of pregnancy.

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