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ROLE OF CHORIONIC GONADOTROPIN IN THE REGULATION OF ANTIGEN-INDEPENDENT DIFFERENTIATION OF IMMUNOCOMPETENT SPLEEN CELLS

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Recently chorionic gonadotropin (CG) has attracted the attention of research workers not only as an important reproductive placental hormone and tumor cell marker [11], but also as an active modulator of immune reactions. It is important to emphasize that processes of reproduction and oncogenesis are closely connected both with each other and with the immune system, for which CG is a mediator of intercellular interactions [5, 6]. The role of gonadotropin, secreted by lymphocytes under mixed culture conditions is not completely clear, but the fact itself indicates the need for its involvement in the immune response. Considering that in the gestation period the hormone persists for a long time in the body not only during pregnancy, but also in the developing fetus, it is important to know what effect it may have on maturation processes of immunocompetent cells, which as a rule take place at the antigen-independent stage of differentiation.

The aim of this investigation was to determine the effect of CG and its action, indirectly through ovarian hormone, on processes of antigen-independent differentiation of the T and B cells of the spleen and on its radioresistant stroma.

EXPERIMENTAL METHOD

Experiments were carried out on 400 male (CBA × C57BL/6)F_1 hybrid mice weighing 20-22 g. Some animals underwent ovariectomy under ether anesthesia. The ovariectomized mice were used in the experiments after 1 month. CG was injected subcutaneously on alternate days for 19 days in doses of 40 or 200 U, which is comparable with the average serum level of the hormone in pregnant women in the 1st and the 2nd and 3rd trimesters respectively.

The experiments were conducted in the following variants: 1) injection of CG into donors of T lymphocytes; 2) injection of CG into donors of B lymphocytes; 3) injection of CG into recipients which then underwent lethal irradiation (219.3 mCi/kg) and restoration by syngeneic splenocytes, together with thymus-dependent antigen, namely sheep's red blood cells (SRBC). T Lymphocytes were obtained by fractionation of splenocytes on a column with nylon fiber [9], and B cells from a suspension of splenocytes after treatment with anti-BaO serum and guinea pig complement. The purity of the fractionated cell populations was estimated in the cytotoxic test with anti-BaO-serum [7] and with EAC-rosette formation [1]. In the population enriched with B cells 0-2% of T lymphocytes was present, whereas in the population enriched with T cells, 1-6% of B lymphocytes was present.

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TABLE 1. Effect of CG on Formation of Precursors of Helper T Lymphocytes in Spleen of Uncastrated and Ovariectomized Female Mice

Group of animals	Experimental action on donors of T cells	Log of number of AFC in recipients spleens	Geometric mean
1	Injection of solvent of		
($n=34$)	hormone	$3,068\pm0,045$	1169,4
2	Injection of CG – 40 U	$2,965\pm0,046$	922,5
($n=20$)	p_{2-1}	$>0,05$	
3	Injection of CG – 200 U	$2,976\pm0,086$	946,2
($n=15$)	p_{3-1}	$>0,05$	
	p_{3-2}	$>0,05$	
4	Ovariectomy, injection of		
($n=11$)	solvent of hormone	$3,011\pm0,053$	1025,6
5	Ovariectomy, injection		
($n=13$)	of CG – 40 U	$3,126\pm0,035$	1336,5
	p_{5-4}	$>0,05$	
6	Ovariectomy, injection		
($n=9$)	of CG – 200 U	$2,995\pm0,072$	988,5
	p_{6-4}	$>0,05$	
	p_{6-5}	$>0,05$	

Legend. n) Number of recipient animals in groups.

TABLE 2. Effect of CG on Functional Activity of Splenic B Lymphocytes of Uncastrated and Ovariectomized Female Mice

Group of animals	Experimental action on donors of B cells	Log of number of AFC in recipients' spleens	Geometric mean
1			
($n=34$)	Injection of solvent of hormone	$3,068\pm0,045$	1169,4
2	Injection of CG – 40 U	$2,884\pm0,062$	765,5
($n=15$)	p_{2-1}	$<0,02$	
3	Injection of CG – 200 μ	$2,775\pm0,068$	595,6
($n=18$)	p_{3-1}	$<0,001$	
		$>0,05$	
4	Ovariectomy, injection of		1025,6
($n=11$)	solvent of hormone		
5	Ovariectomy, injec-		
($n=13$)	tion of CG – 40 U	$3,358\pm0,051$	2280,3
	p_{5-4}	$<0,001$	
6	Ovariectomy, injection		
($n=10$)	of CG – 200 U	$2,942\pm0,069$	874,9
	p_{6-4}	$>0,05$	
	p_{6-5}	$<0,001$	

T or B lymphocytes from donors treated with CG were transferred into syngeneic, lethally irradiated recipients together with B or T lymphocytes of intact donors respectively in the ratio of 1:1 (total concentration $2\cdot10^7$ cells), and $2\cdot10^8$ SRBC were injected in a parallel experiment. Functional activity of B or T cells was estimated in a syngeneic transfer system [10], by recording the level of the adoptive immune response on the 5th day by the direct method of local hemolysis in agarose gel [8].

The results were subjected to statistical analysis by Student's t test. In all cases, the logarithm of the number of antibody-forming cells (AFC) served as the basis for comparison.

EXPERIMENTAL RESULTS

Injection of CG into noncastrated and ovariectomized donors of T lymphocytes had no significant effect on the level of the adoptive immune response (Table 1). Since in this model the height of the immune response depends on activity of precursors of helper T lymphocytes [10], whose formation took place at the antigen-independent stage in noncastrated and ovariectomized females, it can be considered that both CG itself and the ovarian sex hormones, responsible for transmitting it. Immunomodulating effects [2, 4], cannot modulate processes of antigen-independent differentiation of helper T cells.

TABLE. 3. Effect of CG on Functional Activity of Cells of Radioresistant Splenic Stroma of Uncastrated and Ovariectomized Animals

Groups of animals	Exptl. action on recipients before irradiation	Log. of number of of AFC in recipients' spleens	Geometric mean
1	Injection of solvent of		
($n=12$)	hormone	$3,167\pm0,069$	1468,9
2	Injection of CG – 40 U	$3,199\pm0,054$	1581,2
($n=12$)	p_{2-1}	$>0,05$	
3	Injection of CG – 200 U	$3,063\pm0,088$	1156,1
($n=12$)	p_{3-1}	$>0,05$	
	p_{3-2}	$>0,05$	
4	Ovariectomy, injection of		
($n=9$)	solvent of hormone		
		$3,644\pm0,083$	4405,5
5	Ovariectomy, injection of		
($n=10$)	CG – 40 U	$3,549\pm0,130$	3539,9
	p_{5-4}	$>0,05$	
6	Ovariectomy, injection of		
($n=7$)	CG – 200 U	$3,604\pm0,113$	4017,9
	p_{6-4}	$>0,05$	
	p_{6-5}	$>0,05$	

Injection of CG into ovariectomized donors of B cells revealed a spontaneous dose-dependent immunostimulating effect of the hormone on maturation of B lymphocytes. Whereas in a dose of 200 U, CG had no significant action, in a dose of 40 U it more than doubled the AFC level. Considering that when functional activity of B lymphocytes was tested the cells were transferred in a strictly limited number, it can be postulated that CG, in a dose characteristic of the 2nd and 3rd trimesters of pregnancy, stimulates antigen-independent differentiation of splenic B lymphocytes. Injection of different doses of the hormone into uncastrated donors of B lymphocytes reduced their ability to form an adoptive immune response equally (Table 2). These results confirm yet again that CG, in uncastrated animals, exerts its immunomodulating effects indirectly through ovarian hormones which, moreover, can completely cancel out the positive control due to a small dose of CG.

Subsequent experiments showed that injection of CG for 19 days into uncastrated or ovariectomized recipients of a mixture of T and B lymphocytes before their irradiation and restoration did not lead to any statistically significant change in the adoptive immune response (Table 3). It can thus be asserted that neither CG itself nor ovarian sex steroids induced by it can modulate activity of the radioresistant stroma of the spleen, involved in presentation of the antigen and capable of regulating the height of the adoptive immune response.

To conclude, it must be emphasized that at the antigen-independent stage of differentiation of immunocompetent spleen cells, only B lymphocytes are subject to hormonal control from both CG and sex steroids. The opposite direction of the immunomodulating effects of these hormones and the existence of close correlation between their secretion, suggest a self-regulating system of endogenous immunocorrection during pregnancy. Considering that the ontogenetic development of the immune system in the fetus as a rule coincides with the 2nd and 3rd trimesters of pregnancy [3], it can be tentatively suggested that CG, which during this period is present also in the blood stream of the fetus, participates in the formation of the humoral stage of its immune system.

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