EFFECT OF CHORIONIC GONADOTROPHIN ON HEMATOPOIETIC STEM CELLS

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Chorionic gonadotrophin (CG) is an important factor in the endocrine regulation of reproduction in mammals [1]. There is convincing evidence of the participation of this hormone in immunodepression during pregnancy [4-6]. However, as a rule CG has been studied in models assessing the function of mature lymphocytes. However, we know that differentiation of lymphocytes begins at the level of hematopoietic stem cells (HSC) [2, 3].

The study of the effect of this hormone on HSC, on the one hand, is thus an important step without which it would be difficult to judge the role of the hormone as a regulator of immunogenesis, and on the other hand, investigations of th is kind are needed because they can widen our knowledge of the neuroendorine control of hematopoiesis.

There are as yet no clear morphological criteria of HSC, and the most acceptable and generally adopted method of their evaluation is accordingly determination of their functional activity, namely their ability to form endogenous or exogenous colonies in lethally irradiated mice, each of which is a clone of the progeny of a single HSC.

The aim of this investigation was to study the efect of CG on the number of colony-forming units (CFU-S) in the spleen and bone marrow and also the possible dependence of the effects of CG on ovarian sex steroids.

EXPERIMENTAL METHOD

Experiments were carried out on 350 male CBA mice weighing 20-22 g. Bilateral ovariectomy was performed on some of the animals 1 month before the experiments began.

CG was injected subcutaneously in doses of 40 or 200 U as a course of 10 injections on alternate days. Thus the animals were subjected to the action of CG for a period of 20 days, which is equal to the duration of pregnancy in mice. The dose of 40 U/mouse was obtained by extrapolating the mean blood CG level in women in the second and third trimesters of pregnancy, whereas the dose of 200 U/mouse was obtained by similarly extrapolating the peak hormone level (8-10 weeks of pregnancy) [8].

The number of CFU-S in the spleen and bone marrow was determined by exogenous colony formation [9]. For this purpose, on the 20th day after injection of CG began, splenic and femoral bone marrow cells were removed from noncastrated and ovariectomized donors and transferred in doses of $5 \cdot 10^5$ and $1 \cdot 10^5$ respectively into lethally irradiated (219.3 mCi/kg) syngeneic recipients. On the 8th day after whole-body irradiation and transplantation, the number of macroscopic colonies was counted in the recipients' spleens.

The following variants of the experiments were undertaken: 1) injection of CG into the mice donating HSC, 2) injection of CG into lethally irradiated recipients. In the first case the effect of CG was studied on the number of stem cells in the hematopoietic organs, whereas in the second case the effect of CG was studied on colony formation and on the radioresistant splenic stroma, which lays the role of specialized niches for exogenous stem cells [2, 3].

The experimental results were subjected to statistical analysis by Student's test.

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TABLE 1. Effect of CG on Number of CFU-S in Bone Marrow of Noncastrated and Ovariectomized Mice

Group No.	Experimental action on donors	Number of CFU-S per 10^5 bone marrow cells	Number of CFU-S per femur
1 (n=17)	Injection of solvent of CG	12,8±0,70	3145,1±402,5
2 (n=11)	Injection of CG (40 U)	10,9±0,90	2896,2±521,6
	p_{2-1}	>0,05	>0,05
3 (n=12)	Injection of CG (200 U)	24,9±1,20	5904,8±958,2
	p_{3-1}	<0,001	<0,05
	p_{3-2}	<0,001	<0,02
4 (n=17)	Ovariectomy, injection of solvent of CG	12,8±0,90	2000,0±118,6
5 (n=15)	Ovariectomy, injection of CG (40 U)	8,8±1,70	1470,8±240,1
	p_{5-4}	<0,05	>0,05
6 (n=15)	Ovariectomy, injection of CG (200 U)	4,0±1,50	525,7±100,6
	p_{6-4}	<0,001	<0,001
	p_{6-5}	<0,05	<0,01

TABLE 2. Effect of CG on Number of CFU-S in Spleen of Noncastrated and Ovariectomized Mice

Group No.	Experimental action on donors	Number of CFU-S per 5 × 10^5 spleen cells	Number of CFU-S per organ
1 (n=12)	Injection of solvent of CG	18,5±2,10	5379,4±530,1
2 (n=11)	Injection of CG (40 U)	12,0±1,60	3348,0±423,0
	p_{2-1}	<0,001	<0,02
3 (n=15)	Injection of CG (200 U)	13,3±1,40	4149,6±284,9
	p_{3-1}	<0,001	>0,05
	p_{3-2}	>0,05	>0,05
4 (n=17)	Ovariectomy, injection of solvent of CG	12,9±0,80	3650,7±263,6
5 (n=14)	Ovariectomy, injection of CG (40 U)	14,0±1,00	4260,0±148,9
	p_{5-4}	>0,05	>0,05
6 (n=17)	Ovariectomy, injection of CG (200 U)	9,7±1,50	2854,5±108,1
	p_{6-4}	>0,05	<0,02
	p_{6-5}	<0,02	<0,001

TABLE 3. Effect of CG on Colony Formation in Spleen of Castrated and Noncastrated Mice, Irradiated and Restored with Intact Syngeneic Bone Marrow Cells

Group No.	Experimental action on recipients	Number of CFU-S in spleen
1 (n=17)	Injection of solvent of CG	12,8±0,70
2 (n=13)	Injection of CG (40 U)	18,3±1,40
	p_{2-1}	<0,01
3 (n=12)	Injection of CG (200 U)	24,1±1,80
	p_{3-1}	<0,01
	p_{3-2}	<0,02
4 (n=19)	Ovariectomy, injection of solvent of CG	11,8±1,00
5 (n=17)	Ovariectomy, injection of CG (40 U)	18,2±1,70
	p_{5-4}	<0,01
6 (n=16)	Ovariectomy, injection of CG (200 U)	14,8±1,50
	p_{6-4}	>0,05
	p_{6-5}	>0,05

EXPERIMENTAL RESULTS

Injection of CG in a does of 40 U into noncastrated donors of bone marrow cells had no significant effect on the number and functional activity of HSC, whereas in a dose of 200 U the hormone increased the number of HSC in the bone marrow, capable of forming exogenous colonies, statistically significantly. After ovariectomy, on the other hand, CG in a dose of 200 U reduced the number of CFU-S statistically significantly. A dose of 40 U

had no such effect on ovariectomized mice (Table 1). CG evidently exerts its action on female mice through sex steroids from the ovaries, while at the same time having an independent and alternative action.

Considering that the CFU-S in the bone marrow are less precommitted than CFU-S in the spleen [7], and also since the splenic and medullary fractions of HSC are closely interlinked and exchange stem cells as a result of migration [2], we studied the effect of CG on CFU-S in the spleen. Table 2 shows that injection of CG in a does of 40 or 200 U into noncastrated donors reduced the relative number of CFU-S statistically significantly, whereas CG depressed their absolute number only in a low dose (40 U). Against the background of ovariectomy CG also reduced the number of HSC in the spleen capable of forming exogenous colonies, but this effect was given by the high dose (200 U) and not by the low dose of CG.

Thus CG, in a dose of 200 U, has an independent depressive action on the pool of bone marrow and splenic HSC, whereas in a does of 40 U it is inactive, but this concentration is evidently sufficient to induce, through the ovaries, endocrine processes leading to a decrease in the absolute number of CFU-S in the spleen. The possibility cannot be ruled out that this effect of the low dose is connected with differences in the sensitivity of CFU-S in the bone marrow and spleen to sex steroids.

Injection of CG in a dose of 40 or 200 U into lethally irradiated ovariectomized recipients of bone marrow cells led to statistically significant and dose-dependent stimulation of colony-forming processes. Injection of CG into ovariectomized recipients also caused an increase in the relative number of CFU-S, but after injection of the low dose of the hormone (Table 3).

The results suggest that in a dose of 40 U CG can give an independent effect in animals with intact ovaries, not mediated through sex steroid hormones. However, the difference between the effects of a high dose of CG in noncastrated and ovariectomized animals suggests more complex relations between CG and the ovaries during colonization of the stromal microenvironment of the spleen.

The investigation thus showed that CG, in a dose corresponding to its peak concentration during pregnancy, has an independent inhibitory action on CFU-S in the spleen and bone marrow. In noncastrated animals, injections of CG in this same dose give a directly opposite effect, due to hypersecretion of sex steroids by the ovaries, which evidently have an alternative action. Injection of CG in a low dose has a positive action on lethally irradiated bone marrow recipients, leading to more active colonization of the spleen. Sex steroids, induced for gonadotrophin secretion also give a similar effect.

CG can be regarded as an important factor in the regulation of immunogenesis at its earliest stages, and reproductive hormones can be regarded as factors regulating hematopoiesis in mammals.

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