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ORIGINAL ARTICLE

Progesterone inhibits embryotoxic effect of the complement system

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Abstract

The complement system of normal human serum (NHS) manifests a strong, dose dependent embryotoxic potential when administered to chick embryos inducing, among others, also malformations of the brain. We have demonstrated, however, that the degree of complement-induced embryotoxicity varied remarkably in the course of the menstrual cycle of fertile healthy women, although the complement serum activity (CH100) exhibited no significant fluctuation. On the other hand, the variation of embryotoxicity appeared negatively associated with progesterone levels. Following our results high progesterone levels occurring physiologically in luteal phase of the menstrual cycle suppress the embryotoxic action of the complement system.

Introduction

A considerable bulk of data supports the idea that complement plays an important role in human reproduction (Vanderpuye et al. 1992). Pathology of the complement system may be responsible for some cases of sterility and spontaneous abortion (Tichenor et al. 1995). Complement regulatory proteins (CRP), which inhibit complement activation through both the alternative and classical pathways, were found in the cell populations of the embryo and maternal reproductive tissues (Oglesby et al. 1996; Holmes et al. 1992). CRP helps to protect the allograft of the conceptus before the attack of maternal immune system and contributes to its survival and normal development within the female reproductive tract (Cunningham and Tichenor 1995). Precise balance among the complement system, its regulatory proteins, receptors, and other mediators in tissues of the reproductive tract is obviously essential for fertilization and embryonic development (Hasty et al. 1994).

Numerous experiments performed in the past have demonstrated the normal human blood serum (NHS) as highly embryotoxic for the chick embryo (Jelínek and Konícková 1975). NHS acts in a dose-dependent manner producing a specific malformation complex called the "straight jacket syndrome" that comprises, among others, also defects of the central nervous system (Jelínek et al. 1976 a, b). The pattern of CNS malformation varies from exencephaly and cerebral herniation, to minor tissue abnormalities with histogenetic and functional consequences. It has been deduced that the factor responsible for the embryotoxic action of NHS was the complement system (Loštický and Jelínek l978) and further described, in some unpublished studies, that the sera embryotoxicity was highly individual and dependent probably also on age and sex. Sera deficient in some components of the complement exerted a significantly lower embryotoxic activity than sera with the complete complement system (Zeman and Nováková 1996). We decided, therefore, to continue studying the NHS embryotoxicity with respect to female reproductive functions.

The aim of our present study was 1) To investigate whether the NHS-induced embryotoxicity varies in the course of menstrual cycle in normally cycling fertile women and, if so, 2) To find out in the same group of women whether the changes in embryotoxicity are positively associated with variation of complement serum levels and (C´) activity.

Material and Methods

Sera were obtained from venous blood of 29 fertile normally cycling Caucasian women aged 18–32 at regular time periods (twice a week). Eight samples per month from one woman were thus obtained. C3, C4 and C1i components of complement were detected in these samples by radioimmunoassay and complement activity (CH100) was measured by haemolysis of antibody sensitized erythrocytes using a haemolytic complement kit from Binding Site, Birmingham, England. The procedures were routinely performed in the laboratory Imumed, Prague, Czech Republic. To identify the course of each menstrual cycle, progesterone levels were followed by radioimmunoassay technique. Spectria Progesterone (^{125}I) kits from Orion Diagnostica, Espoo, Finland, were employed for this purpose in the Biochemical Laboratory of the Institute of Mother and Child Care, Prague.

Sera obtained from 5 women aged 20–26 exhibiting regular cycle were used for the further study. As an experimental object we employed the chick embryo on incubation day 4 (staged 20–24 following Hamburger and Hamilton 1951). Fertile eggs of White Leghorn random bred stock were purchased from Dominant, farm Dobrenice, and incubated at 37.5°C in a thermostatic oven. After candling and opening eggs with the common window technique, 3μL of NHS were injected intra-amniotically under the binocular preparation microscope using a glass microcanule with an obliquely ground tip. Each experimental group consisted approximately of 20 externally normal specimens. The windowed eggs were closed with glass slides allowing easy control, sealed with paraffin and reincubated for 4 consecutive days. After checking the embryos everyday, they were harvested on incubation day 8, inspected and dissected under the preparation microscope. The embryotoxicity index (Es), comprising survival time and severity of malformations detected (Jelínek and Konícková 1975), was calculated for each group. Acquired values were plotted and related to each other during the menstrual cycle in each individual case. Statistical evaluation (basic statistics and multiple regression analysis) was performed using the STATISTICA (Statsoft) program.

Results

Measured complement components and also complement activity remained within the physiological range of values during the whole menstrual cycle, exhibiting no significant fluctuations (Fig. 1). No associations were found between the complement haemolytic activity CH100 and the serum components C3, C4 and C1i, on the one hand, and particular phase of menstrual cycle, on the other hand.

On the contrary, Es exhibited significant fluctuations during the menstrual cycle (Table 1, Fig. *2*). Es maintained constant levels during the follicular phase when progesterone levels were low. A fast

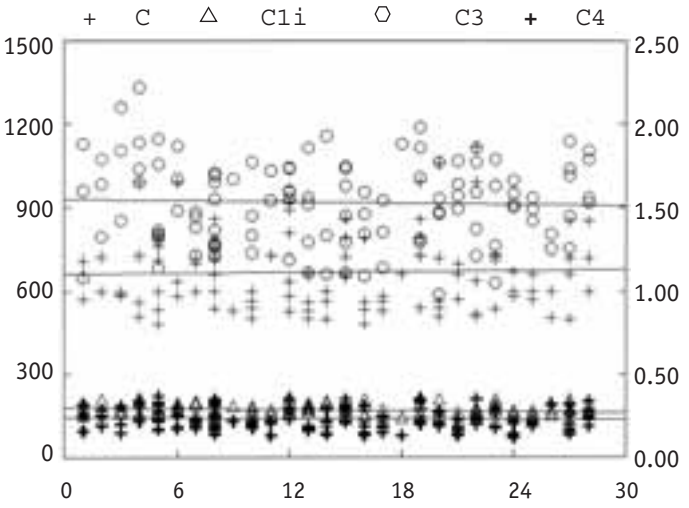

Figure 1. C100. C1i, C3 and C4 during MC

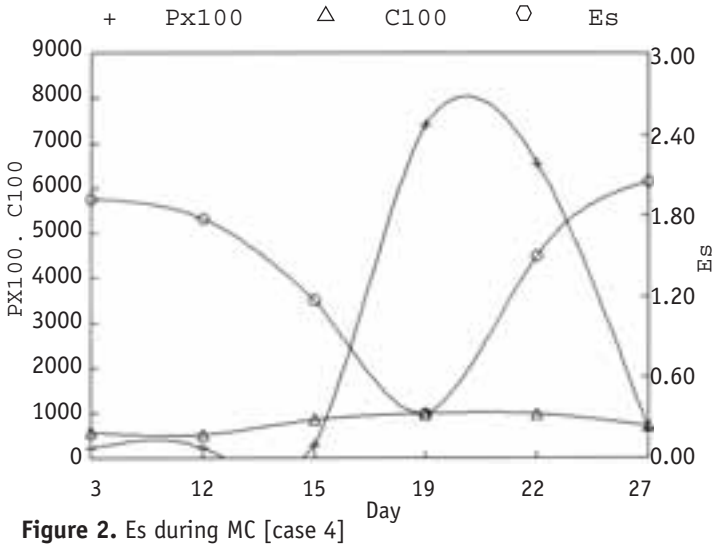

Figure 2. Es during MC [case 4]

decrease of Es at the beginning of the luteal phase corresponded to the increase of progesterone produced by corpus luteum. Simultaneously with corpus luteum involution at the end of the cycle Es attains the original levels.

Discussion

Our original expectation that the degree of NHS embryotoxicity may vary with C´ activity during menstrual cycle failed to be confirmed. The results however indicate that provided that complement levels remained stable, NHS embryotoxicity for the chick embryo was inversely dependent on the level of progesterone. High levels of progesterone suppressed the embryotoxic action of NHS. This stays in accordance with the well-known fact that progesterone acts as a potent immunosuppressant (Kincl and Ciaccio 1980). The mechanism of suppression of C´ embryotoxic effect needs to be further analyzed.

Complement and its related proteins stay in the center of interest of many laboratories investigating the problems of reproductive immunology. Synthesis of the C´ components was described in tissues of the reproductive system (Hasty et al. 1994; Isaacson et al. 1989) depending on hormonal regulation. CRP at the fetal-maternal interface inhibits the complement mediated attack to allogenic tissues of the conceptus (Cunningham and Tichenor 1995; Holmes et al. 1992). The implementation of these relations into

Table 1. Parameters during menstrual cycle

CASE 1	Day of MC	1	5	8	12	15	19	24	28	
	Progesterone	3.53	2.48	2.19	3.18	1.97	3.08	35.08	13.9	nmol/l
	C100	520	570	530	480	440	470	530	590	units/ml
	Es	1.7		2.66		2.82	2.83	2.5	2.6	
CASE 2	Day of MC	2	6	9	13	16	20	23	31	
	Progesterone	3.21	2.45	2.04	2.51	2.29	4.83	17.11	9.35	nmol/l
	C100	530	570	590	530	520	590	530	510	units/ml
	Es		2		2.22	2.5	2.6	2.3	2.83	
CASE 3	Day of MC	4	6	10	13	17	20	24	27	
	Progesterone	1.37	0.92	1.56	3.34	33.5	57.4	25.15	4.13	nmol/l
	C100	520	550	520	520	520	510	520	510	units/ml
	Es		2		2.36	2.83	2.77	2.14	2.43	
CASE 4	Day of MC	3	5	8	12	15	19	22	27	
	Progesterone	2.28	2.15	2.68	2.34	2.82	74.2	65.62	6.46	nmol/l
	C100	583	714	600	526	860	990	990	720	units/ml
	Es	1.92			1.77	1.17	0.33	1.5	2.05	
CASE 5	Day of MC	1	5	8	12	15	20	23	28	
	Progesterone	2.54	2.88	2.13	2.56	2.94	29.7	45.43	18.8	nmol/l
	C100	570	790	860	916	860	508	726	850	units/ml
	Es	2.67	2.47			2.42	1.53	2.31	2.4	

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the mechanisms of teratogenesis requires further experiments targeted to the molecular mechanisms of the complement-mediated embryotoxicity and the chick embryo model may prove useful even in these types of investigations.

Our study brings at least further evidence that the immune and endocrine systems act in close co-operation and separating their functions from each other seems hardly possible.

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