Arch Gynecol Obstet (1989) 244: 185–191

Archives of
Gynecology
and Obstetrics

Originals

The Relation Between Serum Sex Steroid Levels and Plasma Cell Infiltrates in Endometritis

R. Punnonen, M. Lehtinen, K. Teisala, R. Aine, I. Rantala, P. K. Heinonen, A. Miettinen, S. Laine, and J. Paavonen

Department of Obstetrics and Gynecology, Tampere University Central Hospital, University of Tampere, Medical School, SF-33520 Tampere, Finland

Summary. We measured serum levels of progesterone and estradiol among 35 patients with endometritis confirmed by endometrial biopsy. The onset of symptoms took place predominantly in the proliferative phase of the cycle. A negative correlation was found between the serum progesterone levels and the histopathologic severity of plasma cell endometritis. Our results suggest that the hormonal status contributes to the immune response and susceptibility to endometrial infection.

Key words: Estradiol – Progesterone – Endometritis – Plasma cells

Introduction

Sex steroid hormones regulate both systemic and local immune responses. Immunoglobulin (Ig) production is higher in females than in males [5], while males tend to have higher cell-mediated responses [25]. T-cells can be found even in the normal endometrium and plasma cells are frequently seen in acute and chronic endometritis [3, 14, 17]. Enhanced incorporation of plasma cells into the endometrial stroma during the estrous phase has been described in animal models [13, 30]. Estradiol causes inhibition of suppressor T-cell activity [19]. Progesterone on the other hand, suppresses polyclonal antibody production *in vitro* [20]. Thus, the sex-steroid hormones may be important modifiers of the inflammatory responses in upper genital tract infections.

The most common cause of pelvic inflammatory disease (PID), *Chlamydia trachomatis* [15], has recently been shown to cause extensive T-cell and plasma cell responses in patients with PID [7, 11]. In the present study we correlated serum estradiol and progesterone levels with the severity of endometrial inflammation among patients with proven PID.

Offprint requests to: R. Punnonen, M.D. (address see above)

Material and Methods

Patients

The study population consisted of 46 patients hospitalized for acute PID. Of these 46 patients eleven used oral contraceptives (OC), and 35 used other methods or none. OC-users were excluded to avoid the confounding effect of exogenous hormone administration. Endometrial biopsy showed plasma cell endometritis in all 35 patients who were eligible.

Histopathologic and Immunohistologic Methods

Endometrial biopsy was obtained during general anesthesia by transcervical aspiration. This method and the grading of endometrial inflammation have been described in detail elsewhere [17]. Briefly, the histopathologic diagnosis of endometritis was based on the identification of plasma cells by methyl green pyronin stained [1]. Endometrial inflammation was defined as mild, moderate or severe. The presence or absence of lymphoid follicles was also recorded.

Endometrial IgG, IgA, and IgM producing plasma cells were identified by an indirect immunoperoxidase staining as described in detail [11, 24]. Briefly, in the first phase of the immunoperoxidase staining, unconjugated rabbit antisera to human Ig:s (IgA, IgG, IgM) (Dakopatts a/s, Denmark) were followed by horseradish peroxidase (HRP-)labeled goat antiserum to rabbit IgG (Cappel Laboratories, USA). Specificity controls were performed as earlier described [24]. The quantity of plasma cells belonging to different subtypes was based on the specific staining of different Ig-classes. Absolute number of plasma cells per 2 mm^2 of endometrial stroma was counted with a semiautomated morphometry system equipped with a graphic table (MOP-3, Kontron, West-Germany).

The plasma cell responses were expressed in four classes corresponding to total quantity of plasma cells/2 mm^2 within a given range: +++ = 3200–600, ++ = 600–280, + = 280–30, − = <30.

Microbiologic Studies

Specimens for isolation of *C. trachomatis* and *Neisseria gonorrhoeae* were obtained from the cervix, endometrium, fallopian tubes, and cul-de-sac. The specimen collection and identification of *C. trachomatis* and *N. gonorrhoeae* were performed as described previously [6].

Serum Estradiol and Progesterone Deteminations

Blood samples were drawn on admission at the same time as the microbiologic and biopsy specimens were obtained. Serum 17B-estradiol concentrations were measured by radioimmunoassay (RIA) (EIR, Switzerland) and serum progesterone concentrations by RIA (Farmos Diagnostics, Turku, Finland).

Statistical analysis were performed by using the BMDP® statistical software. Analysis of variance was used for the demonstration of statistically significant trends.

Results

Onset of Symptoms and Isolation of Micro-Organisms During Menstrual Cycle

In four cases histological discrimination between the proliferative and secretory phases of the endometrium was impossible due to severe inflammation (3 cases with *C. trachomatis* and 1 case with *C. trachomatis* and *N. gonorrhoeae*). Symptoms began (as recalled by the patient) during the proliferative phase of the menstrual cycle in 29 (83%) of the 35 cases, and during the secretory phase

Table 1. Relation between phase of menstrual cycle, onset of symptoms and isolation of micro-organisms from the endometrium

Patient category	Number of patients		
	Onset of symptoms	Isolated micro-organisms	
		C. trachomatis	*N. gonorrheae*
Proliferative phase	29*/35	7/24	3/24
Secretory phase	6*/35	1/7	0/7

* $P < 0.01$

Table 2. Relation between mean serum estradiol and serum progesterone levels (nmol/l) and severity of plasma cell endometritis

Endometritis	No. of patients	Serum Estradiol (E)	Serum Progesterone (P)	E/P ratio × 100
All patients				
Mild	13	0.21 ± 0.05	8.2 ± 1.6[a]	3.0 ± 0.4[b]
Moderate	6	0.43 ± 0.13	4.7 ± 1.5	10.7 ± 2.3
Severe	16	0.34 ± 0.06	4.2 ± 0.7	10.8 ± 2.0
Patients in the proliferative phase only				
Mild	9	0.24 ± 0.06	7.1 ± 1.9	3.6 ± 0.4
Moderate	6	0.43 ± 0.13	4.7 ± 1.5	10.7 ± 2.3
Severe	9	0.33 ± 0.07	4.5 ± 1.1	10.5 ± 2.8

The value are mean ± standard error; [a] $P \leq 0.05$; [b] $P \leq 0.01$.

in 6 (17%) cases (Table 1). Of the latter six patients, five had low serum progesterone levels at the time of the diagnosis suggesting anovulatory cycles or luteral phase defect.

N. gonorrhoeae was isolated from the endometrium in three out of 24 (13%) patients seen during a histopathologically proven proliferative phase but from none seen during the secretory phase of the cycle. There was a two-fold difference in the endometrial isolation rates of *C. trachomatis* between the two phases (Table 1).

Correlation of Estradiol and Progesterone Levels with the Severity of Endometritis and Salpingitis

Serum progesterone but not estradiol levels showed a significant negative correlaion with the severity of plasmas cell endometritis. The mean progesterone level was two times higher and the estradiol/progesterone (E/P) ratio three times lower in mild disease compared to moderate or severe disease ($P = 0.05$ and 0.01, respectively, Table 2). This was true also when only patients seen in

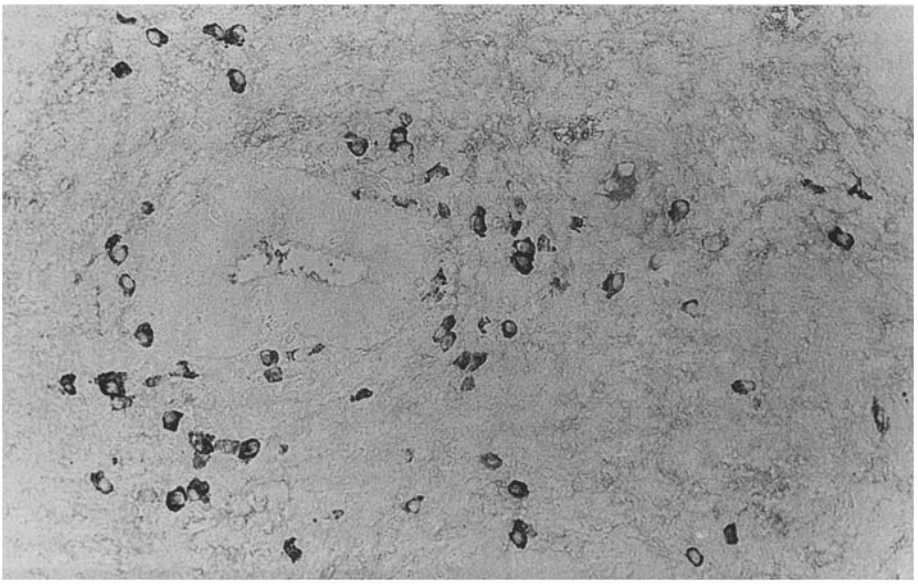

Fig. 1. Moderate endometritis with pronounced IgA-class plasma cell infiltration. (× 578, HRP conjugated anti-human IgA)

the proliferative phase were analyzed. Also lymphoid follicles were seen only during the proliferative phase of the menstrual cycle (Table 1).

Correlation of Estradiol and Progesterone Levels with the Density of Endometrial Plasma Cells

Endometrial plasma cell response consisted of IgG and/or IgA producing plasma cells, which formed dense infiltrates in the endometrial stroma (Fig. 1). IgM plasma cells were seen only occasionally (data not shown). Patients with most abundant infiltrations (+++) of IgG plasma cells had 2- to 3-fold higher absolute estradiol levels than the other patients. The quantity of IgA plasma cells showed a statistically significant ($P < 0.05$) correlation with serum estradiol but not with progesterone levels (Table 3). Overall, the total plasma cell count showed a good correlation with the serum E/P ratio ($P < 0.01$).

Discussion

In most of our patients the onset of symptoms occurred during the proliferative phase, the main two micro-organisms isolated from the genital tract in all but four cases being *C. trachomatis* and *N. gonorrhoeae* [6, 18]. *C. trachomatis* and *N. gonorrhoeae* were isolated from the endometrium in a majority of the cases and mostly during the proliferative phase of the cycle. In a recent study women

Table 3. Relation between serum estradiol and serum progesterone levels (nmol/l) and relative numbers of total IgG- or IgA-producing plasma cells in inflamed endometrium

Total plsma cell count	No. of patients	Serum Estradiol (E)	Serum Progesterone (P)	E/P ratio × 100
+	10	0.18 ± 0.08	8.0 ± 1.7[a]	3.0 ± 1.5[b]
++	14	0.37 ± 0.13	5.9 ± 1.7	8.7 ± 3.0
+++	11	0.34 ± 0.15	3.5 ± 0.9	11.2 ± 3.9
IgA plasms cell count				
+	18	0.15 ± 0.11	5.3 ± 1.4	7.5 ± 2.0
++	7	0.16 ± 0.21	3.1 ± 1.0	8.6 ± 2.5
+++	4	0.38 ± 0.16	4.5 ± 0.5	8.5 ± 3.3
IgA plasma cell count				
+	12	0.27 ± 0.07[a]	6.3 ± 1.6	7.5 ± 2.0
++	5	0.33 ± 0.08	4.1 ± 1.9	16.2 ± 3.0
+++	4	0.42 ± 0.17	5.3 ± 0.4	7.8 ± 3.0

The values are means ± standard error; [a] $P \leq 0.05$; [b] $P \leq 0.01$

who reported the onset of symptoms early in the cycle had either chlamydial or gonococcal PID whereas women with nongonococcal – nonchlamydial PID had the onset of symptoms usually in the luteal phase [26]. *N. gomorrhoeae* is isolated more often early in the cycle than late in the cycle [4]. Because only one of our patients had ovulatory serum progesterone levels anovulation may also predispose it the PID.

A trend towards increasing serum estradiol levels was seen in the endometritis patients as the severity of endometrial inflammation increased. The trend was found to be most pronounced when the IgA-plasma cell response was considered. Further more lymphoid follicle formation was noted in the endometrial stroma during the proliferative phase of the cycle only. In animal models estrogens recruit plasma cell progenitors from the mesenteric lymph nodes into the endometrial mucosa [13]. Extrogen administration increases the levels of local IgG and IgA in the rat endometrium [30]. However, experimental data emphasizes the role of exogeneous estrogen administration in the prolongation of genital chlamydial infection [22, 23]. Thus, it could be argued that in PID the estrogen enhanced plasma cell response may be inapproriate or even harmful.

We found a negative correlation between the severity of the plasma cell endometritis and serum progesterone levels. Progesterone antagonizes certain effects of estrogen by reducing the levels of estrogen receptors within the cell [8, 9]. Although many experimental animal and in vitro models support the inhibitory effect of progesterone on the bactericidal and immunological activities of the uterus [5, 12, 28] some are against [21]. Furthermore progesterone administration is able to rescue mice from disseminated gonococcal infection [10]. Thus, progesterone may slow down the overwhelming plasma cell response in PID, but may also exert a positive effect on the restriction of the microbial invasion.

Intrauterine device (IUD) is able to cause chronic endometritis. However, we were not able to find major differences in the sex-steroid hormone/plasma cell relationship in women who had an IUD compared to women with barrier type contraception (data not shown). On the contrary, OC-users usually had a mild endometritis and were excluded from the study.

Previous studies have suggested that oral contraceptives protect against acute PID and that among women with proven PID OC-users usually have mild PID [26, 27]. Our results indicate that the circulating progesterone and estrogen levels affect the susceptibility of the upper genital tract to microbial in vasion. They also affect the immune response in the endometrium by regulating the degree of plasma cell recruitment (estrogen) and polyclonal antibody production (estrogen and progesterone). Thus, greater emphasis should be placed on the interactions between reproductive and immune systems.

Acknowledgements. The excellent technical assistance of Mrs. Helena Eljander is gratefully acknowledged. This work was supported by grants from the Academy of Finland (J.P.) and the Emil Aaltonen Foundation (M.L.).

References

1. Ahlqvist J, Andersson L (1972) Methyl green-pyronin staining: Effects of fixation; use in routine pathology. Stain Technol 47:17–22
2. Chipperfield EJ, Evans BA (1975) Effect of local infection and oral contraception on immunoglobulin levels in cervical mucus. Infect Immun 11:215–221
3. Crum CP, Egawa K, Fenoglio CM, Richart RM (1983) Chronic endometritis: the role of immunohistochemistry in the detection of plasma cells. Am J Obstet Gynecol 147:812–815
4. Eschenbach DA, Harnisch JP, Holmes KK (1977) Pathogenesis of acute pelvic inflammatory disease: role of contraception and other risk factors. Am J Obstet Gynecol 128:838–850
5. Grossman CJ (1985) Interactions between the gonadal steroids and the immune system. Science 227:257–259
6. Heinonen PK, Teisala K, Punnonen R, Miettinen A, Lehtinen M, Paavonen J (1985) Anatomic sites of upper genital tract infection. Obstet Gynecol 66:384–389
7. Hallberg T, Wölner-Hanssen P, Mårdh P-A (1985) Pelvic inflammatory disease in patients infected with Chlamydia trachomatis: in vitro cell mediated immune response to chlamydial antigens. Genitourin Med 61:247–251
8. Hsueh AJW, Peck EJ, Clark JH (1976) Progesterone antagonism of the oestrogen receptor and oestrogeninduced uterine growth. Nature (London) 254:337–339
9. Hsueh AJW, Peck EJ, Clark JH (1976) Control of uterine estrogen receptor levels by progesterone. Endocrinology 98:438–444
10. Kita E, Takahashi S, Yasui K, Kashiba S (1985) Effect of estrogen (17β-estradiol) on the susceptibility of mice to disseminated gonococcal infection. Inf Immun 49:238–243
11. Lehtinen M, Rantala I, Aine R, Miettinen A, Laine S, Heinonen P, Peisala K, Punnonen R, Paavonen J (1986) B-cell response in Chlamydia trachomatis endometritis. Eur J Clin Microbiol 5:596–598
12. Matsuda H, Okuda K, Fukui K, Kamata Y (1985) Inhibitory effect of estradiol-17β and progesterone on bactericidal activity in uteri of rabbits infected with Escherichia coli. Infect Immun 48:652–657
13. McDermott MR, Clark DA, Bienenstock J (1980) Evidence for a common mucosal immunologic system, II. Influence of the estrous cycle on B immunoblast migration into genital and intestinal tissue. J Immunol 124:2536–2539
14. Morris H, Edwards J, Tiltman A, Emms M (1985) Endometrial lymphoid tissue: an immunohistological study. J Clin Pathol 38:644–652

15. Mårdth P-A, Ripa KT, Svensson I, Weström L (1977) Chlamydia trachomatis infection in patients with acute salpingitis. N Engl J Med 296:1377–1379
16. Paavonen J, Vesterinen E, Mayer B, Saksela E (1982) Colposcopic and histologic findings in cervical chlamydial infection. Obstet Gynecol 59:712–714
17. Paavonen J, Aine R, Teisala K, Heinonen PK, Lehtinen M, Miettinen A, Punnonen R, Grönroos P (1985) Chlamydial endometritis. J Clin Pathol 38:726–732
18. Paavonen J, Teisala K, Heinonen PK, Aine R, Laine S, Lehtinen M, Miettinen A, Punnonen R, Grönroos P (1987) Microbiological and histopathological findings in acute pelvic inflammatory disease. Br J Obstet Gynecol 94:454–460
19. Paavonen T, Andersson LC, Adlercreutz H (1981) Sex hormone regulation of in vitro immune response. Estradiol enhance human B-cell maturation via inhibition of suppressor T-cells in pokeweed mitogen-stimulated cuiltures. J Exp Med 154:1935–1941
20. Paavonen T (1985) Hormonal regulation of human lymphocyte functions *in vitro*. Academic Dissertation, University of Helsinki
21. Pasley JN, Rank RG, Hough AJ, Cohen C, Barron AL (1985) Absence of progesterone effects on chlamydial genital infection in female guinea pigs. Sex Transm Dis 12:155–158
22. Pasley JN, Rank RG, Hough AJ Jr, Cohen C, Barron Al (1985) Effects of varying doses of estradiol on chlamydial genital infection in ovariectomized guinea pigs. Sex Transm Dis 12:8–13
23. Rank RG, White HJ, Hough AJ, Pasley JN, Barrow AL (1982) Effects of estradiol on chlamydial genital infection of female guinea pigs. Infect Immun 38, 699–705
24. Rantala I, Mäki M, Laasonen A, Visakorpi JK (1985) Periodate-lysine-paraformaldehyde (PLP) as the only fixative for the study of duodenal mucosa. Morphologic and immunohistochemical results at light and electron microscopic levels. Acta Pathol Microbiol Immunol Scand 93:165–172
25. Santoli D, Trinchieri G, Zmijewski CM, Koprowski H (1976) HLA-related control of spontaneous and antibody-dependent cell-mediated cytotoxic activity in humans. J Immunol 117:765–769
26. Sweet RL, Blankfort-Doyle M, Robbie MO, Schacter J (1986) The occurence of chlamycial and gonococcal salpingitis during the menstrual cycle. JAMA 255:2062–2064
27. Svensson L, Weström L, Mårdh PA (1984) Contraceptives and acute salpingitis. JAMA 251:2553–2555
28. Tuffrey M, Taylor-Robinson D (1981) Progesterone as a key factor in the development of a mouse model for genital-tract infection with chlamycia trachomatis. FEMS Microbiol Lett 12:111–115
29. Washington AE, Gove S, Schachter J, Sweet RL (1985) Oral contraceptives, Chlamydia trachomatis infection and pelvic inflammatory disease. A word of caution about protection. JAMA 253:2246–2250
30. Wira CR, Sandoe CP (1977) Sex steroid hormone regulation of IgA and IgG in rat uterine secretions. Nature (London) 268:534–536

Received October 7, 1988/Accepted February 28, 1989