Hormonal regulation of complement components and receptors throughout the menstrual cycle

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OBJECTIVES: Complement components have been recently demonstrated to be present in the reproductive tract. Among these components, C3 synthesis by glandular epithelium of the rat uterus has been shown to be regulated by estrogen; progesterone inhibits this synthesis. However, the hormonal regulation of C3 and the presence of other complement factors in the human has not been investigated to date. In this study we examined the presence and the hormonal regulation of different complement components and receptors in the human endometrium at various phases of the menstrual cycle of normally cycling women with no pelvic pathologic abnormalities.

STUDY DESIGN: Endometrial tissue was obtained from normally cycling women, and immunohistochemistry was performed by means of monoclonal antibodies against C3, factors B, decay-accelerating factor, membrane cofactor protein, and complement receptor types 1, 2, and 3. The tissue was incubated with minimal essential media without methionine containing methionine labeled with sulfur 35. Immunoprecipitations were performed on the media with goat antihuman C3 antibody, and the proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

RESULTS: C3 was found to be present in the glandular epithelial cells of luteal endometrium. Biosynthesis as analyzed by immunoprecipitation with anti-C3 antibody was found to increase during the luteal phase of the cycle and to be minimal in the proliferative phase. Like C3, factor B and decay-accelerating factor were localized to the luteal glandular epithelial cells. In contrast, membrane cofactor protein was found to be present in the glandular epithelium throughout the menstrual cycle, and complement receptor type 1 was present only in the stromal compartment of luteal endometrium. Complement receptor type 3 was present only in the infiltrating leukocytes in the luteal endometrium, whereas complement receptor type 2 was undetectable.

CONCLUSIONS: These findings suggest that several components of the complement system exist in the human endometrium in a hormone-dependent manner and may play a role in normal reproductive function. (Am J Obstet Gynecol 1994;170:168-75.)

Key words: Complement, menstrual cycle

Estradiol and progesterone induce profound alterations, including growth and differentiation of the glandular epithelial cells, stromal cells, fibroblasts, and granulocytes, in the biochemistry and histologic characteristics of the human endometrium. These predictable cyclic changes include the secretion of various proteins. The function and regulation of different secretory proteins are of major importance in understanding the role of the endometrium in successful reproduction. One of these secretory proteins in the rat is complement component C3, which is hormonally regulated. Estradiol administration stimulates the synthesis of C3, whereas simultaneous or delayed administration of progesterone inhibits the synthesis of this protein. This same pattern of hormonal regulation is exhibited in the estrous cycle of the rat because the synthesis of C3 is maximal in estrus and diminishes in metestrus and diestrus.

The presence of complement components in the human reproductive tract has been recently demonstrated. Weed and Arquembourg, and Bartosik and Viscarello by means of immunostaining demonstrated the presence of C3 in the endometrial glands of patients with endometriosis. Both of these studies attributed the presence of C3 in the endometriotic implants and endometrium to deposition from serum. However,
recent work from our laboratory has demonstrated that C3 does not arise from deposition but rather by de novo synthesis. The synthesis and secretion of C3 was found in both endometriotic implants and in the proliferative endometrium of patients with endometriosis. Other complement proteins detected in reproductive tissues include mannose cofactor protein, a regulator of complement activation. It was found to be present on acrosome-reacted human sperm and trophoblast. Additional studies of the polymorphisms of the membrane cofactor protein gene have suggested an association between polymorphisms and recurrent spontaneous abortion. The role of complement C3 and its binding proteins in reproduction has yet to be determined. The third component of complement plays a central role in both the classic and alternative pathways of complement activation and participates in phagocytic and immunoregulatory processes. The activation and regulation of each of these pathways requires the presence of other complement proteins, several of which interact with C3. Activation of the alternative pathway requires the presence of factor B, factor D, and properdin. On the cell surface complement regulation is achieved by proteins such as membrane cofactor protein and decay-accelerating factor, both of which interact with C3 and either participate in the C3b inactivation (mannose cofactor protein) or dissociate the alternative pathway convertase (decay-accelerating factor). Many of the biologic functions of complement are mediated by the interaction of various cleavage fragments of C3 with specific cell membrane receptors such as complement receptor types 1, 2, and 3. To further understand the role complement may play in human reproduction, we studied the presence of C3 and its binding proteins throughout the menstrual cycle.

Material and methods

Patients. Endometrial tissues was obtained from normally cycling women of reproductive age (range 19 to 45 years old). The acquisition of tissue was approved by the University of Pennsylvania Investigational Committee on the Study of Human Beings. All patients were white or black, except for one Asian patient. Luteal endometrial biopsies were obtained in an infertility office from patients with subsequently documented normal pregnancies. Endometrial specimens were also obtained from patients undergoing hysterectomies and laparoscopies in which no pelvic pathologic abnormality was found. The patient had not received exogenous hormones within the previous 6 months. The cycle day of the specimen was determined by endometrial dating according to the criteria of Noyes et al. Ten specimens were obtained during the proliferative phase (days 5 to 14) and 14 from the early to midsecretory phase (days 15 to 23) and 20 from late secretory (day 24 plus). Two patients who had received exogenous progesterone therapy for a minimum of 4 months were also studied. Reagents and antibodies. Minimal essential media was purchased from Sigma (St. Louis) and [14C]methionine (1200 Ci/mmol) from Amersham (Arlington Heights, Ill.). Goat antihuman C3 antibody was obtained from Organon Teknika (West Chester, Pa.). Insoluble protein A (Staph A) was purchased from Sigma. Mouse monoclonal antibodies to factor B were produced with standard protocol. Antibody decay-accelerating factor (IA-10) was a donation from Kinoshita, and antibody to C3 (S11) was obtained from Ouadel. Antibody to membrane cofactor protein (TRA 2-10) was a gift from Peter Andrews. Antibody to complement receptor types 1, 2 (HB5), and 3 (OKM1) were purchased from American Tissue Culture Collection. Antibodies to complement receptor type 2 (OKB7) was obtained from Ortho. Vectastain and diaminobenzidine kits from Vector were used for immunohistochemistry.

Tissue specimens and in vitro incubations. Endometrial biopsy specimens from normally cycling women at various phases of the menstrual cycle were obtained. A portion of the tissue was incubated in minimal essential media without methionine containing 50 μCi/ml [14C]methionine for 12 to 16 hours at 37°C in 5% carbon dioxide and 95% air. The media were collected and centrifuged to remove cellular debris, and the supernatant was stored at −80°C until further analysis. An additional portion of tissue was frozen and used for immunohistochemistry. Additionally, paraffin-embedded formalin and Bouin's fixed tissue sections were immunostained.

Immunoprecipitations. Aliquots of medium containing 100,000 trichloroacetic acid–precipitable counts were added to 50 μl of insoluble protein A (Staph A) and incubated at 4°C for 1 hour. This mixture was centrifuged, and the supernatant was added to 15 μl of goat antihuman C3 antibody or immunoglobulin G and incubated overnight at 4°C. Insoluble protein A (50 μl) was added and incubated for 2 hours at 4°C. The protein A antibody-antigen complex was washed twice with Tris-buffered saline solution, pH 7.5, containing 0.05% sodium dodecyl sulfate/0.3% Tween and once with Tris-buffered saline solution. The antigen was dissociated by incubation with double-strength final sample buffer (Tris-Hydrochloric acid [pH 6.8]/20% glycerol/4% sodium dodecyl sulfate) for 10 minutes at 95°C. The proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis as previously described.
and complement receptor types 1, 2, and 3, which were previously described. Briefly, endometrial samples were fixed in Bouin's or formalin, and embedded in paraffin, and tissue blocks were sectioned by microtome. Tissue sections were deparaffinized with consecutive washes with xylene, absolute ethanol, and tap water. To block endogenous peroxidase, the tissues were incubated in methanol–0.6% to 1.2% hydrogen peroxide solution for 15 minutes. The slides were washed in phosphate-buffered saline solution for 5 minutes followed by phosphate-buffered saline solution–0.1% bovine serum albumin for 5 minutes. Blocking of nonspecific binding was accomplished with normal horse serum (1.5%) for 20 minutes at 37°C. Sections were incubated with the primary antibody at 37°C for 1 hour each. The secondary biotinylated antimouse antibody was added to the slides at a dilution of 1:250 for 30 minutes, followed by phosphate-buffered saline solution and phosphate-buffered saline solution–0.1% bovine serum albumin washes as above. The avidin-biotin complex was added to each section for 45 minutes at 37°C, and the slides were washed in phosphate-buffered saline solution for 10 minutes, followed by a 10-minute wash in phosphate-buffered saline solution–0.1% bovine serum albumin. Chromagen (0.05% diaminobenzidine) was added to each section for 5 minutes, and the slides were washed in running water for 2 minutes. Counterstaining was accomplished by incubation with hematoxylin for 30 seconds, followed by a running tap water wash. The sections were decolorized for 20 seconds with 4% acetic acid and washed in distilled water. The sections were placed in a bath of lithium carbonate for 30 seconds, followed by graded ethanol and xylene baths three times for each 2 minutes. Final sections were mounted with Cytoseal mounting media and examined under light microscopy. The staining of frozen sections was
Table 1. Presence of complement components in endometrial tissues during menstrual cycle

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<th>Proliferative</th>
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<td>C3</td>
<td>–</td>
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<tr>
<td>Factor B</td>
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<td>Membrane cofactor protein</td>
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<td>Decay-accelerating factor</td>
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gl: Glandular epithelium; str, stromal compartment.

performed as in fixed tissue with the exclusion of deparaffinization, and the counterstaining was performed with methyl green.

Results

C3 biosynthesis by human endometrium. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of proteins secreted by human endometrium indicated that one of the major proteins was that with a molecular weight of 180 kDa. This protein was identified by immunoprecipitation as the third component of complement. The production of C3 was greater by luteal endometrium compared with proliferative (Figs. 1 through 3). Interestingly, C3 production was found to be high in patients receiving medroxyprogesterone acetate therapy (Fig. 3).

Localization of complement-binding proteins in human endometrium. The presence of C3 and other complement factors and receptors was analyzed by immunostaining of fixed and frozen tissue sections of each biopsy or hysterectomy specimen. The results are summarized in Table 1. Our results show the presence of C3 in the glandular epithelial cells throughout the luteal phase but not in the proliferative phase (Fig. 4). These findings correlate with the results of our immunoprecipitation of C3 throughout the cycle. Reactivity with anti-factor B antibody was detected only in the glandular epithelial cells of the luteal phase (Fig. 5). Decay-accelerating factor was shown to be present only along the apical surfaces of the glandular epithelial cells in the luteal biopsies (Fig. 6).

Membrane cofactor protein, in contrast to C3, factor B, and decay-accelerating factor, was found to be present in the glandular epithelium throughout the menstrual cycle, thus suggesting that this component is not hormonally regulated (Fig. 7). Results of this analysis of complement receptor using frozen sections indicate that complement receptor type 1 was present in the stromal compartment of the luteal tissue (Fig. 8). The anti–complement receptor type 1 antibody did not react on paraffin-embedded, fixed sections, and the frozen sections do not provide as good a resolution. Complement receptor type 2 was not detected using HB5 and ORB7 antibodies, and complement receptor type 3 was detected only in the infiltrating cells in the luteal endometrium (data not shown).

Comment

The results of these studies demonstrate a cycle-specific appearance of complement C3 and several binding proteins in human endometrium. C3, and factor B, which participates in the initiation and feedback amplification of the alternative pathway, is localized to the glandular epithelial cells of luteal endometrium. In addition, a known regulator of complement activation, decay-accelerating factor, was also localized to the glandular epithelial cells of luteal sections.

Our demonstration of C3 synthesis by the epithelial cells of the luteal endometrium is in good agreement with the findings of Weed and Arquembourg and Bischof et al. The synthesis and presence of C3 in the progesterone-dominant phase of the menstrual cycle raises the possibility that in the human the expression of this particular protein may be regulated by proges-
terone. This concept is further supported by the increased C3 synthesis in patients receiving exogenous progesterone. Currently, however, the inability of polyclonal anti-C3 antibody to detect C3 in luteal endometrium is of interest, and further investigation is required to clarify if a confirmational difference exists between serum C3 and that produced by these cells.

The apparent progesterone regulation in human endometrium and the estradiol regulation in the rat is interesting but not unexpected, because this pattern of regulation has been found in other proteins such as uterine 17β-hydroxysteroid dehydrogenase. This protein was found to be localized to glandular epithelial cells in early to midluteal phase sections and is regulated by progesterone. Although progesterone appears to play a role in the regulation of C3 synthesis by the glandular epithelial cells, the underlying molecular mechanisms are now known at present. It is possible that other inflammatory mediators play a role in this process. Mediators such as interleukin-1 have been demonstrated to be regulated by progesterone, and cellular levels of interleukin-1 messenger ribonucleic acids and serum levels of interleukin-1 are increased during the secretory phase in human endometrium.

A direct action of progesterone on the C3 gene expression, however, has been suggested by the presence of a progesterone response element on this gene. To this context, of interest is the recent observation of a selective regulation of pulmonary epithelial cells by glucocorticoids. As published previously, dexamethasone did not increase C3 synthesis by rat endometrium, whether a differential regulation is a species-specific phenomenon has yet to be determined.

The function of C3 produced by cells of the reproductive tract is unknown at present. Serum C3 has been shown to participate in numerous interactions with other complement components and complement receptors that culminate in inflammatory host-defense
Fig. 6. Immunohistochemical localization of decay-accelerating factor on fixed human endometrium (dilution 1:100). A, Proliferative phase (decay-accelerating factor) (control proliferative phase as in Fig. 1); B, luteal phase (day 24) (decay-accelerating factor); C, luteal phase (day 24) (decay-accelerating factor) (control luteal phase as in Fig. 4). (Original magnification: B, ×40; C, ×10.)

Fig. 7. Immunohistochemical localization of membrane cofactor protein (dilution 1:20). A, Proliferative phase (fixed) (membrane cofactor protein) (control proliferative phase as in Fig. 1); B, luteal endometrium (frozen technique) (membrane cofactor protein); C, luteal endometrium (control).

Fig. 8. Immunohistochemical analysis of frozen human endometrial sections with anti–complement receptor type 1 antibody (dilution 1:500). A, Proliferative phase (complement receptor type 1); B, proliferative phase (control); C, luteal phase (complement receptor type 1) (control luteal phase as in Fig. 7).
and immunoregulatory processes. Therefore the presence of factor B, decay-accelerating factor, membrane cofactor protein, and complement receptor type 1 within this tissue suggest a functional complement system. The concurrent expression of C3, factor B, decay-accelerating factor, and membrane cofactor protein in the same cell type suggests that lytic functions of complement are directed to different targets. Whether the C3 produced by these cells acts in an autocrine fashion in unlikely because receptors for C3 have not been found on these glandular epithelial cells. The differential regulation of component components by these cells suggests a tight control of whatever functions complement may play in the endometrium.

The role of complement in human reproduction has yet to be defined. The differential expression of other complement components such as membrane cofactor protein and decay-accelerating factor in reproductive tissues suggests a tissue-specific function for these proteins. For instance, in trophoblast decay-accelerating factor may play an important role in protecting the semialloimmune fetus from maternal complement-mediated attack. On the other hand, the presence of membrane cofactor protein on only acrosome-reacted sperm may play a role in the fusion process. Another complement component participating in this egg-sperm fusion has been recently described as C1q.

The presence of complement receptor type 1, another regulator of complement activation, in stromal cells implies a protective function for these particular cells. The absence of complement receptor type 2, which has been found to mediate growth of B cells, tends to exclude the possibility of C3 mediating growth of the endometrium. Finally, the expression of complement receptor type 3 was detected only in infiltrating cells.

In conclusion, in this study we have demonstrated the synthesis of complement component C3 by human endometrium and its regulation during the menstrual cycle. Also, we have shown that C3-associated binding proteins that participate in complement activation are present within the human endometrium and their cycle-specific expression suggests their necessity in normal reproductive function.

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REFERENCES
An \( L \)-arginine–nitric oxide–cyclic guanosine monophosphate system exists in the uterus and inhibits contractility during pregnancy

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** OBJECTIVE:** Nitric oxide is synthesized from \( L \)-arginine and it causes relaxation of smooth muscle by elevating cyclic guanosine monophosphate levels. We hypothesized that an \( L \)-arginine–nitric oxide–cGMP system is present in the uterus and modulates contractility.

** STUDY DESIGN:** Isometric tension of the uterus was measured in vitro from pregnant rats in response to various agents that modulate nitric oxide–cyclic guanosine monophosphate production or action.

** RESULTS:** Major findings are as follows: (1) The substrate and a donor of nitric oxide produced uterine relaxation; (2) inhibitors of the nitric oxide–cyclic guanosine monophosphate pathway blocked the relaxation responses; (3) nitric oxide synthase was localized to several uterine cell types; (4) nitric oxide was produced by the uterus during periods when \( L \)-arginine was consumed and citrulline levels increased; (5) effects of nitric oxide substrate on relaxation were mimicked by cyclic guanosine monophosphate; (6) nitric oxide–cyclic guanosine monophosphate responses were decreased during delivery; (7) \( L \)-arginine responses were increased by progesterone, and antiprogestosterone treatment decreased cyclic guanosine monophosphate–induced relaxations.

** CONCLUSION:** An \( L \)-arginine–nitric oxide–cyclic guanosine monophosphate system is present in the uterus and it may regulate relaxation during pregnancy. The inhibitory action of \( L \)-arginine and \( \beta \)-bromo-cyclic guanosine monophosphate was considerably lower during delivery and post partum, indicating that the nitric oxide system may contribute to the maintenance of uterine quiescence during pregnancy, when progesterone levels are elevated, but not during delivery. (Am J Obstet Gynecol 1993;170:175-85.)

** Key words:** Nitric oxide, uterus, contractility, pregnancy, labor

One of the most exciting recent advances in biology and medicine is the discovery that nitric oxide is produced by many cell types and that it is involved in a variety of important cellular processes, including regulation of vascular tone, platelet aggregation, neurotransmission, and immune activation. Nitric oxide is an important mediator of relaxation of various smooth muscles including vascular and gastrointestinal. Nitric oxide is synthesized by the oxidative deamination of a guanidino nitrogen of \( L \)-arginine by at least two different isoforms of a flavin-containing enzyme, nitric oxide synthase. Enzymes from brain and macrophages, representing constitutive and inducible forms, have recently been purified and the genes have been cloned. Synthesis of nitric oxide has been shown to be inhibited by analogs of \( L \)-arginine; \( N^\alpha \)-nitro-\( L \)-arginine