Hormonal Regulation of Complement Factor B in Human Endometrium

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PROBLEM: Recent investigations have demonstrated the presence of complement components in human endometrium in a cycle-specific manner. Luteal phase endometrium has been shown to synthesize complement C3 de novo, whereas proliferative endometrium produces little or no C3. Likewise factor B, which is critical to the activation of the alternative pathway of complement, has been shown to be present only in the glandular epithelium of luteal phase endometrium. This investigation was designed to determine if factor B is present in the endometrium in a high progesterone state such as pregnancy or with exogenous progesterone treatment.

METHOD: Endometrial biopsies were obtained from patients on progesterone therapy. The endometrium of early pregnancy was evaluated by obtaining biopsies from patients with ectopic pregnancies as well as from patients undergoing therapeutic termination. Immunohistochemistry was performed on each biopsy using monoclonal antibodies to factor B.

RESULTS: Our results demonstrate the presence of factor B in the glandular epithelial cells of the endometrium of patients treated with exogenous progesterone therapy. Additionally, factor B was localized to the glandular compartment of the endometrium from patients with ectopic gestations. Interestingly, the evaluation of an implantation site from an early gestation demonstrated factor B in the maternal decidua only; trophoblast did not exhibit the presence of factor B.

CONCLUSION: Factor B exists in the endometrium in a hormone-dependent manner and is not expressed in lethal tissue in early gestation.

INTRODUCTION

The link between reproduction and the immune system is becoming more clearly defined. Most notably, recent investigations involving the complement proteins have demonstrated a relationship between these systems. Complement C3 occupies a central role in both the classical and alternative pathways of complement activation and is also involved in immunoregulatory processes. Activation and regulation of both pathways requires the participation of other key complement components such as factor B, which is critical to the activation of the alternative pathway. On the cell surface, complement regulation is achieved by proteins such as membrane cofactor protein (MCP) and decay

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accelerating factor (DAF), which participate in the inactivation of C3b (MCP) or dissociate the alternative pathway convertase C3bBb (DAF).

Several complement components have been studied with regard to human reproduction. Tissues that express these proteins include human sperm, placenta, and endometrium. Human sperm express MCP, DAF, and CD59.\textsuperscript{5,6} MCP as well as DAF has also been found to be expressed by human trophoblast.\textsuperscript{7,8} Interestingly, certain polymorphisms of the MCP gene appear to be associated with recurrent pregnancy loss.\textsuperscript{9} Furthermore, an additional complement binding protein C1q has been suggested to participate in egg-sperm fusion.\textsuperscript{10}

The importance of an immunocompetent endometrium is the subject of many ongoing investigations. The hormonal regulation of the central component of the complement pathway (C3) in the endometrium was initially studied using the rat uterus. Estradiol administration stimulates the synthesis of rat C3 whereas simultaneous or delayed administration of progesterone inhibits the synthesis of this protein.\textsuperscript{11-13} In the human, however, C3 in the endometrium appears to be upregulated in the progesterone-dominant phase of the menstrual cycle. Like C3 and DAF, factor B (as shown in this article) is localized to luteal glandular epithelial cells\textsuperscript{12} (Fig. 1). In contrast, the regulatory protein MCP was found to be present in the glandular epithelium throughout the menstrual cycle. This study demonstrates that factor B, a key activator of complement, is present in the endometrium in high progesterone states such as pregnancy and exogenous progesterone therapy.

Fig. 1. Localization of factor E using immunohistochemistry in luteal endometrium of a patient with normal pelvic findings. One micro-liter of anti-factor B antibody was diluted with 100 μl of PBS/BSA.

MATERIALS AND METHODS

Patients
Endometrial tissue was obtained from seven patients who received exogenous progesterone treatment for at least four months. Endometrial tissue was also obtained from four patients with documented ectopic gestations as well as two patients undergoing therapeutic termination. This study was approved by the Human Investigations Committee of Emory University and the University of Pennsylvania where these studies were conducted. Informed consent was waived by each committee.

Detection of factor B in endometrial tissue
Immunohistochemistry was performed using a mouse monoclonal antibody against human factor B (J.D. Lambris, unpublished data). Endometrial samples were fixed in Bouin’s or formalin, imbedded in paraffin, and tissue blocks 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 a methanol/0.6 to 1.2% hydrogen peroxide solution for 15 min. The slides were washed in phosphate buffered saline (PBS) for 5 min followed by PBS/0.1% bovine serum albumin (BSA) for 5 min. Blocking of non-specific binding was accomplished with normal horse serum (1.5%) for 20 min at 37°C. Sections were incubated with the primary antibody (1 to 10 μg/ml) at 37°C for 1 h. The secondary biotinylated anti-mouse antibody (Vector Laboratories) was added to the slides at a dilution of 1:250 for 30 min, followed by PBS and PBS/0.1% BSA washes as above. Avidin-biotin complex was added to each section for 45 min at 37°C and the slides were washed in PBS for 10 min, followed by a 10-min wash in PBS/0.1% BSA. Chromagen (0.05% DAB) was added to each section for 5 min and the slides were washed in distilled water for 2 min. Counterstaining was accomplished by incubation with hematoxylin for 30 seconds, followed by a distilled water wash. The sections were dehydrated 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 each for 2 min. Final sections were mounted using Cytoseal mounting media and examined under light microscopy. The staining of frozen sections was performed as in fixed tissue with the exclusion of deparaffinization, and the counterstaining was performed with methyl green.
RESULTS

Previous studies have indicated that some of the complement components are upregulated during the luteal phase of the cycle. These results suggest that progesterone may be responsible for the expression of these components. Immunohistochemical evaluation of endometrium from patients receiving exogenous progesterone clearly demonstrate the presence of factor B in the glandular epithelial cells (Fig. 2). In support of the view that progesterone may be a mediator of factor B expression, we found that factor B was present in the glandular compartment of the endometrium from patients with ectopic gestations (Fig. 3). The examination of an implantation site from an early gestation demonstrated factor B to be present in the maternal decidua. Factor B could not be detected in trophoblastic tissue (Fig. 4).
DISCUSSION

Factor B is a single-chain, 93,000-dalton, B-globulin zymogen serine protease. The simultaneous expression of factor B and C3 suggest that a functionally active complement system is present. Findings in the mouse model indicate that complement activation is present in uterine secretions on days 2 and 3 of pregnancy.\textsuperscript{15} Whether or not complement activation is necessary for successful implantation is yet to be determined. Therefore, we chose to study the response of the endometrium to hormones of pregnancy in the situation of an ectopic gestation. Additional information was derived from tissue from therapeutic terminations. The results of these studies indicate that both C3 and its activator, factor B, are present in the same cell type: glandular epithelium. The formation of the complex C3bBb is essential for the subsequent cleavage of C3 and is the initial step in the activation of the alternative pathway. The localization of factor B and C3 to the same cell type is therefore not surprising and suggests that activation is now possible. Based on immunohistochemical findings, our data would suggest that factor B and C3 are upregulated or more highly expressed by conditions of high serum progesterone levels. The molecular mechanisms underlying this stimulated expression are currently unknown; whether this expression is a direct action of progesterone on the C3 and factor B gene is not known. Interestingly, however, a potential progesterone response element has been identified on the human C3 gene.\textsuperscript{16} It is also possible that this regulation can require both estrogen and progesterone or may also represent an indirect action of progesterone through stimulation of various cytokines. Again, further studies are required to examine the possibilities. These preliminary studies appear to indicate that a functionally active complement system is present at the time of implantation and early gestation. However, an exact role, if any, of these complement components has yet to be determined.

CONCLUSION

In conclusion, the presence of factor B in the endometrium appears to be hormonally regulated. Its detection in high progesterone states raises the possibility that this protein may have a role in implantation and maintenance of early pregnancy. Future studies of the hormonal regulation of factor B gene expression may be crucial to our understanding of the immunologic response of pregnancy.

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REFERENCES


