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MHC-CLASS II CELLS IN THE OVIDUCT PLAY A ROLE DURING THE MENSTRUAL CYCLE AND EARLY PREGNANCY?

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SUMMARY

The epithelium of the oviduct may play significant roles in defending oviductal tissue and fertilized ovum from infection. The aim of this study was to determine the role of MHC-class II cells by analyzing them via HLA-DR immunoreactivity in the human oviduct epithelium during the menstrual cycle and early pregnancy. Oviducts from 21 healthy women undergoing tubal sterilization were collected and fixed in 10% formalin. After dehydration in ethanol, the oviducts were embedded in paraffin. Sections were immunostained with monoclonal mouse-anti human HLA-DR antibody, and immunoreactivity was determined using immunoperoxidase technique under light microscopy. While increased HLA-DR immunoreactivity was observed in columnar epithelial cells during the proliferatory phase of the menstrual cycle, immunoreactivity was withdrawn from the epithelium in the secretory phase and in early pregnancy. These results indicated that human oviduct epithelium has positive immunoreactivity for MHC-II+ cells and that increased immunoreactivity correlated with ovulation. Varied distribution of HLA-DR immunoreactivity in the oviduct epithelium may play a significant role in the regulation of local immunocompetence during the menstrual cycle. Increased HLA-DR immunoreactivity in the proliferatory phase may be needed against invading microorganisms. At the same time, a decrease in HLA-DR immunoreactivity in the secretory phase and early pregnancy may be required to endure preimplantation of the foreign embryo.

Key Words: *Oviduct, HLA-DR, İmmunohistochemistry,*

ÖZET

TUBA UTERİNADAKİ MHC-Class II HÜCRELERİNİN, MENSTRUAL SİKLUS VE ERKEN GEBELİK DÖNEMLERİNDEKİ ROLÜ

Tuba uterina lümenini döşeyen epitel dokusu, bu bölgeye gelebilecek patojenlere karşı ilk bariyeri oluşturmaktadır. Doku uygunluk kompleksi-MHC (Major Histocompatibility Kompleks) class II hücrelerinin, bu bölgede varlığının gösterilmesi lokal immün cevap sırasında, tuba uterinanın antijen sunucu olarak rol oynayabileceğini düşündürmektedir.

Bu çalışmada, menstrual siklus ve erken gebelik döneminde insan tuba uterina epitelindeki doku uygunluk kompleksi-II hücrelerinin rolünün, HLA-DR immunoreaktivitesi ile değerlendirilmesi amaçlanmıştır. Tuba uterina örnekleri, 21 sağlıklı kadından, mini laparotomi ile Pomeroy usulü yapılan tüp ligasyonunda alınarak % 10 formalinde tesbit edildi. Alkol serileri ile dehidrate edildikten sonra, ksilende şeffaflandırılan dokular parafin bloklara gömüldü. İmmünohistokimyasal boyama için, 6 mikron kalınlığındaki kesitler deparafinize edildi. Anti HLA-DR birincil antikoru uygulandıktan sonra, immunoperoksidaz tekniği kullanılarak immünoreaktivite ışık mikroskobu altında değerlendirildi.

Tuba uterinanın tek sıralı prizmatik epitel hücrelerinde, menstrual siklusun proliferasyon fazında HLA-DR immunoreaktivitesi belirgin pozitif olarak gözlenirken, sekresyon fazında ve erken gebelik döneminde ise immunoreaktivitenin azaldığı saptandı. Bu çalışma sonucunda, tuba uterinanın ovulasyon öncesi dönemde positif HLA-DR immünoreaktivitesinin mikroorganizmalara karşı gerekli immün cevabın sağlanmasında; ovulasyon sonrası dönemde azalmış immunoreaktivitenin ise implantasyon öncesi yabancı olan embriyonun optimal yaşaması ve korunmasına yönelik gerekli lokal immünokompetansın sağlanmasında önemli rol oynayabileceği düşünülmüştür.

Anahtar Kelimeler: *Tuba Uterina, HLA-DR, Menstrual Siklus*

The oviduct, which conveys secondary oocytes from the ovaries to the body of the uterus, is a dynamic and cyclically changing structure. Cyclical fluctuations in the circulating levels of estradiol and progesterone are responsible for cyclical and structural changes of the

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oviduct (1). The oviduct contains multiple cell types. This cellular diversity assists in the maturation and transportation of gametes and fertilized ovum and in the early development of the embryo (2).

The epithelium is the first protective barrier against foreign pathogens invading the human oviduct (2). These pathogens are blocked by initializing local immunity, which is greatly affected by sexual maturation and ovarian steroids (3). Local immunity may arise from Major Histocompatibility Complex (MHC) class II proteins. Human Leukocyte Antigens (HLA-DR, -DQ, -DP), which are members of the MHC-class II group, are transmembranous glycoproteins with a central role in cell-to-cell interactions in the initiation of immune response, especially in the early phase of T lymphocyte activation. Constitutive expression of MHC-class II antigens has been identified in a limited number of cells, mainly bone marrow-derived cells such as macrophages, B lymphocytes, monocytes, dendritic cells and Langerhans' cells (4).

The female reproductive tract represents an environment in which the physiological need for controlling MHC-class II antigens may be particularly delicate. Different needs for immunocompetence can be assumed to be at hand during different phases of the normal menstrual cycle (5).

The purpose of this study was to investigate the immune role of the oviduct by assessing HLA-DR immunoreactivity throughout the menstrual cycle and early pregnancy.

MATERIALS AND METHODS

Isthmic portions of oviducts were obtained from 21 healthy women undergoing tubal ligation by the Pomeroy technique in the Department of Family Planning of the Aegean Social Insurance Institute, Obstetric & Gynecology Hospital. Tubal ligation was performed, with patient consent, in conjunction with the termination of unwanted pregnancies.

Subjects were classified into three groups: (i) proliferatory phase (5th-14th day of cycle, n=7), (ii) secretory phase (15th-28th day of cycle, n=7) and (iii) early pregnancy (6th-7th week of gestation, n=7). At the same time, histology of endometrial biopsies was obtained and histologic dating of the endometrium performed, as described by Noyes et al (1950). The mean age of patients was 34±5 years (age range: 29-39). All patients had regular menstrual cycles, and there were no significant differences among the average ages of the women in the three groups. None were receiving any hormones or medication likely to interfere with ovulation.

Tissue samples were preserved in 10% formaline solution for evaluation under light microscopy. Samples were embedded in paraffin blocks after dehydration with graded ethanol. Paraffin sections (5(m) were deparaffinized with xylene and rehydrated through a graded ethanol series.

Indirect immunoperoxidase was used for immunohistochemistry. Sections were washed with phosphate-buffered saline (PBS) and treated with 0.1% trypsin solution. They were then washed with PBS and pre-treated with 0.3% hydrogen peroxide for 10 minutes at room temperature to inactivate endogenous peroxidase activity. They were washed again in PBS and incubated overnight with the primary antibody (1:100 dilution monoclonal mouse anti-human HLA-DR antibody - DAKO-M 0746) in a humidity chamber at 4(C. They were then incubated with anti-mouse immunoperoxidase antibody (Universal Dako LSAB2 Kit). Color reaction was developed using a Dako AEC Substrate System (Dako) containing 3-amino-9-ethylcarbazole. Sections were counterstained with Mayer's hematoxylin and covered with mounting medium. Normal mouse serum was used for immunoreactivity on negative control sections.

HLA-DR staining was graded semi-quantitatively according to the following scale: strong staining intensity (+++); moderate staining intensity (++); weak staining intensity (+); absence of staining (-).

RESULTS

HLA-DR immunoreactivity in the oviduct

TABLE 1: Summary of HLA-DR distribution in oviduct epithelium during menstrual cycle and early pregnancy

	Proliferatory Phase	Secretory Phase	Early Pregnancy Stage
Oviduct epithelium	+ ++	++	+

epithelium according to menstrual cycle and early pregnancy is summarized in Table 1. Positive HLA-DR immunoreactivity was detected in the epithelial layer and the lamina propria of the oviduct in all phases of the menstrual cycle and early pregnancy, but the intensity of immunoreactivity differed during these phases.

In the proliferatory phase, intense immunostaining was detected in columnar epithelial cells, especially in epithelial cell cytoplasm and under the epithelial layer (Figure 1-A). In this phase, HLA-DR+ cells were found in the epithelium and in the lamina propria, especially near the capillaries. Observation was significantly better under higher magnification (Figure 1-B). An increase in immunoreactivity was detected during the proliferatory phase. Immunoreactivity decreased after ovulation, but HLA-DR+ cells were observed in the intraepithelial region in the beginning of this phase (Figure 1-C). Weak immunoreactivity of the cytoplasm of epithelial cells was still detected during the secretory phase (Figure 1-D).

In the specimens taken during early pregnancy, immunoreactivity was detected in the oviduct epithelium, with HLA-DR distribution similar to that of the secretory phase

(Figure 1-E). Weak immunoreactivity was seen only in the cytoplasm of the epithelial cells.

There was no immune staining in the controls (Figure 1-F).

DISCUSSION

In this study, positive HLA-DR immunoreactivity in the human oviduct, which exhibited MHC-class II antigens, was observed during the menstrual cycle and early pregnancy. However, HLA-DR immunoreactivity in the oviduct epithelium showed some differentiation throughout the menstrual cycle. While strong HLA-DR immunoreactivity was detected in the columnar

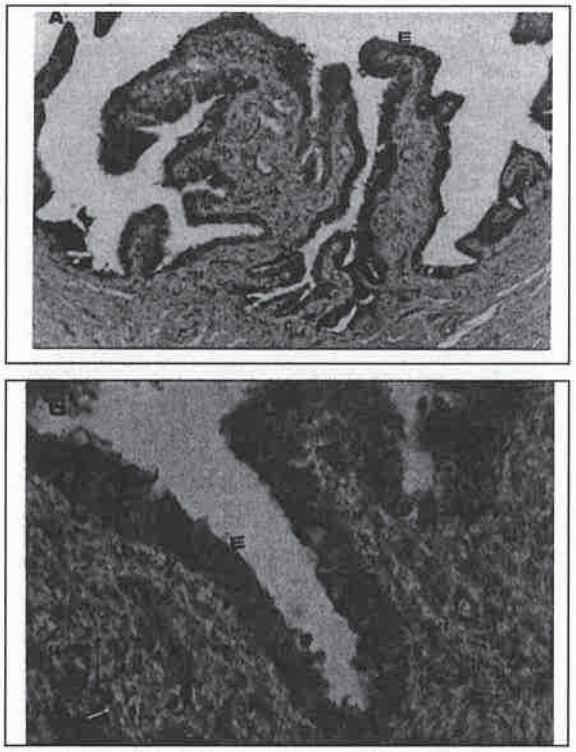

FIGURE 1:

A, B: Immunohistochemical localization of HLA-DR in oviduct during proliferatory phase. Immunoperoxidase staining for HLA-DR in the oviduct, showing strong staining in the tube epithelium. There were increased HLA-DR+ cells in the lamina propria in the proliferatory phase of the menstrual cycle. Immunoreactivity was detected especially in epithelial cell cytoplasm and under the epithelial layer. Immunoreactivity was observed better in higher magnification during the proliferatory phase. (A) X100, (B) X400 (Original magnification).

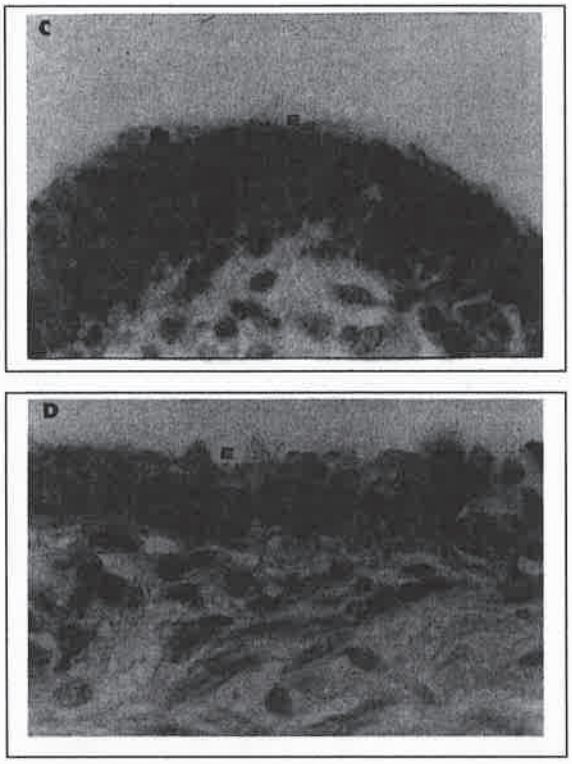

FIGURE 1:

C, D: Immunohistochemical localization of HLA-DR in human oviduct during secretory phase. Immunoreactivity decreased after ovulation, but HLA-DR+ cells were seen in the intraepithelial region in the beginning of this phase (C). Weak immunoreactivity was still detected in the cytoplasm of epithelial cells during the secretory phase (D). (C,D) X1000 (Original magnification).

FIGURE 1:

E: Immunohistochemical localization of HLA-DR in oviduct during early pregnancy. Decreased immunoreactivity was detected in the epithelial layer, and this staining was observed in the cytoplasm of epithelial cells . X1000 (Original magnification).

FIGURE 1:

F: Control immunostaining for HLA-DR. In the control section, there was no immune staining. X1000 (Original magnification). E: Oviduct epithelium

epithelium in the proliferatory phase, this immunoreactivity was withdrawn in the secretory phase.

In addition to its role in transporting and supporting the nutrition of the fertilized ovum, the oviduct plays an important role in the early division and differentiation of the embryo. This study also extends the characterization of the local immune system in the oviduct. Detection of MHC-class II cells in normal non-lymphoid tissues such as breast and endometrial epithelium suggests that the expression and secretion of these cells during the menstrual cycle may be under hormonal control (6). A number of studies on different animals species also indicated that immune cells played an important role in cyclical ovarian activity (7-11). These studies also emphasized that HLA-DR+ cells can show changes throughout the menstrual cycle. Bulmer and Earl previously described MHC-class II antigen expression in the columnar epithelium of the human oviduct (6). Hormonally mediated regula-

tion was suggested, based on the differences in class II antigen expression in the epithelium of oviducts in pregnant and non-pregnant women. Edelstam et al demonstrated that cyclical MHC-class II antigen variation in the oviduct may indicate hormonal regulation of class II antigen synthesis (5).

Strong HLA-DR immunoreactivity in the proliferatory phase may be required for the response against foreign infective microorganisms. In contrast, decreased HLA-DR immunoreactivity in the secretory phase might provide more optimal conditions for the immunologically foreign spermatozoa and fertilized ovum to escape from potential MHC-class II cells that are restricted by the mother's immune system.

The reduction in HLA-DR immunoreactivity in the secretory phase would be reflected in an impaired ability to recognize and respond to pathogens. As a result, Chlamydial infections, the major cause of acute salpingitis, usually start in the secretory phase (12). Strong MHC-class II antigen expressions in the secretory phase could lead to various kinds of inflammatory diseases. This would lead to the secretion of inflammatory mediators in the local environment, such as (-IF, which might counteract the normal secretory reduction of class II antigen expressions in the oviduct (5).

While there were no specific changes of Ig-positive cells in the non-infected oviduct in the different menstrual cycle phases, intraepithelial lymphocytes were detected (13). The lymphocytes in the human oviduct consist exclusively of T suppressors (CD8+). The secretion of these T lymphocytes is unresponsive to sperm antigens, although Ig-positive cells, especially IgA and IgG, are detectable against sperm antigens (13). These results suggested that intraepithelial lymphocytes may normally function in assisting in the induction of immune tolerance to sperm antigens in the oviduct. In addition, HLA-DR immunoreactivity at the fimbrial portion of the oviduct was found with T lymphocytes in fertile cases. In infertile cases, immunoreactivity was strongly stained in the epithelium, and T lymphocytes increased proportionally. These results lead us to believe that infertility occurs when there is high immunological response. In another study that aimed to investigate the protective effects of the oviduct against microorganisms, E Coli was injected into the oviduct lumen. The results showed that the human oviduct epithelium exhibits endocytic properties towards luminal soluble and particle antigens that are unrelated to MHC-class II expression and menstrual cycle phase (2). Oviducts prohibit the transportation of microorganisms from the vagina to the abdomen, which also provides protection against foreign bodies through immune-system reactivity of antigen-presenting cells.

In addition to the observation of MHC-class II cells in the oviduct epithelium during the menstrual cycle, macrophages were also detected using specific antibodies for human macrophages (PM-1K and PM-2K) (12). When human macrophage immunoreactivity was observed to be +++, well-developed macrophages were also detected (14). It is possible that such macrophages might be involved in the physiological functions of the tubes during the reproductive period. While large numbers of macrophages were present in both ectopic and intrauterine pregnancy tissue, occasional macrophages were identified in the oviduct walls of non-pregnant women. Local immune modulation of maternal cytotoxicity, in response to foreign fetal antigens, may revolve around the production of soluble suppressor factors by macrophages that down-regulate the activities of other immuno-component cells. Oviduct epithelium does not show morphological changes in ectopic pregnancy, nor is there loss of MHC-class I surface antigens, although changes in expression of HLA-D locus products have been described (15).

Bulmer and Earl observed that oviducts of non-pregnant women had a variable number of epithelial cells labeled for HLA-DR (6). The relative proportions of DR-Positive and DR-Negative epithelium showed no obvious relation to the stage of the menstrual cycle. In the early pregnancy stage, the oviduct epithelium showed uniform intense reactivity for HLA-DR. These results

suggest differential regulation of class II- MHC immunoreactivity in tube epithelial cells, possibly mediated by hormones and/or a trophoblast product.

Oviduct and endometrium epithelial cells are capable of different responses to a given stimulus; in intrauterine pregnancy, the oviduct epithelium is uniformly class II- MHC+, whereas endometrial gland epithelium is essentially negative for HLA-D locus antigens (6). Thus, a difference in HLA-DR distribution in the oviduct epithelium during the menstrual cycle and/or early pregnancy stage may be caused by differences in hormonal regulation and/or secreted proteins. However, the role of MHC-class II antigens is still unclear.

Based on our own and previous findings, we can suggest that if immune cells and their products are involved in cyclical differences, there must be a trigger that activates the immune cells. The MHC-class II cells play a central role in regulating the immune response, the effect of which is limited by lymphocytes and antigen-presenting cells such as macrophages and B cells. Their positive immunoreactivity indicates increased immune response in the oviduct. These results show that the oviduct, with the help of its antigen-presenting cells, has the important role of producing an immune response against microorganisms before ovulation, supplying the necessary local immunocompetence for the survival and post-ovulation protection of the embryo, which is regarded as a foreign body before implantation.

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