Expression of class I human leukocyte antigen (HLA) and β2-microglobulin is associated with decidualization of human endometrial stromal cells

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Human leukocyte antigen (HLA) class I molecules play a central role in the immune system through either presentation of endogenous antigen and activation of T lymphocytes or functional emergence of natural killer (NK) cells. Various types of immune cells are present in the human endometrium and are believed to be involved in reproductive function and/or immunological reaction. However, little is known about the expression status and function of HLA class I molecules in the human endometrium. We therefore examined mRNA expression of HLA class I. In addition, we analysed gene expression and localization of β2-microglobulin (β2-MG), which is the non-variant chain of all HLA class I molecules. Compared with non-decidualized tissues, mRNA expression of both HLA class I and β2-MG was significantly higher in decidualized endometria in the late secretory phase, under progestin treatment and during early pregnancy. Immunohistochemical studies revealed that β2-MG was localized in decidualized endometrial stromal cells, indicating that the distribution of β2-MG is topologically correlated with that of CD56bright+ NK cells. In-vitro culture of human endometrial stromal cells demonstrated that HLA class I mRNA was induced during the decidualization by progesterone. Accordingly, the expression of HLA class I molecules is transcriptionally activated along with decidualization of human endometrial stromal cells, and may represent an immuno-endocrine function of the endometrium.

Key words: β2-microglobulin/decidualization/endometrial stroma/HLA class I/NK cell

Introduction

Human endometrium consists of various kinds of immune cells as well as glandular epithelial cells and endometrial stromal cells (ESC). Immune cells in the endometrium include macrophages, T lymphocytes and large granular lymphocytes which express the natural killer (NK) cell marker CD56, but there are few B lymphocytes and plasma cells (Bulmer, 1994, 1995). It has recently been suggested that immune cells interact with endometrial glandular cells, ESC, and presumably with trophoblasts, and play an important role in the modulation of the function of these cells (Tabibzadeh, 1991) and the implantation process (Saito et al., 1993).

Activated T lymphocytes produce cytokines which mediate both cellular and humoral immune reactions. Molecules of the major histocompatibility complex (MHC) play a fundamental role in immune response, since antigen presentation by MHC molecules is necessary for activation of T lymphocytes (Janeway and Travers, 1994). The human MHC molecules, also known as HLA, comprise two molecules, HLA class I and HLA class II. Tabibzadeh et al. (1988) reported that T lymphocytes might induce HLA class II expression and inhibit proliferation of the endometrial glandular cells. Although HLA class I molecules are immunohistochemically localized in the human endometrium (Johnson and Bulmer, 1984), their quantitative changes and functional aspects have not been elucidated.

In the present study, therefore, we investigated the level of expression of HLA class I mRNA in the human endometrium throughout the menstrual cycle and during early pregnancy. We also examined β2-microglobulin (β2-MG) mRNA expression, which constitutes the non-variant chain of all HLA class I molecules, in the same specimens. Expression and localization of β2-MG protein and its topological relevance to CD56bright+ NK cells were analysed by immunohistochemistry. Furthermore, the induction of HLA class I gene expression in ESC was examined using an in-vitro model of decidualization.

Materials and methods

Specimens

Human endometrial tissues were obtained from 22 women, aged 27–51 years, who underwent hysterectomy for the treatment of cervical intraepithelial neoplasia. At the time of operation, 16 women had had regular menstrual cycles: five in the proliferative phase, four in the early secretory phase, three in the mid-secretory phase and four in the late secretory phase. Four women had been medicated with oestrogen–progestin combined pills (EP) to prolong the occurrence of menstruation for the prevention of severe anaemia. The remaining two women were pregnant in 8 and 12 weeks of gestation respectively. Decidual tissues in the first trimester of pregnancy from eight women who underwent legal elective abortion were saved for immunohistochemical staining for β2-MG. Informed consent was obtained from each patient according to the Guidelines (No. 91) of The Ethical Committee of Kyoto University, Faculty of Medicine. The tissue specimens from which RNA was extracted were immediately frozen in liquid nitrogen and stored at –80°C until use. Tissues subjected to immunohistochemical staining were fixed in 10% (v/v) formalin and embedded in paraffin in a vacuum automatic tissue processor (Sakura...
RNA isolation and Northern blot analysis

Total RNA from the frozen tissues and cultured cells was isolated with Trizol Reagent (Life Technologies, Gaithersburg, MD, USA). An HLA class I (B7) clone (Sood et al., 1981) which can hybridize with HLA-A and C mRNA as well as HLA-B mRNA (Coppin et al., 1985) was obtained through the Japanese Cancer Research Resources Bank (Tokyo, Japan). A probe for the β2-MG gene was cloned by reverse transcription-polymerase chain reaction described previously (Arao et al., 1994). Northern blot hybridization was performed as follows. Briefly, total RNA (5 mg) was separated by electrophoresis in a 1% agarose-formaldehyde gel and transferred onto a nylon membrane. The membrane was prehybridized for 1 h at 65°C in 5×SSPE, 1% sodium dodecyl sulphate (SDS), 5×Denhardt’s solution, and 0.5 mg/dl salmon sperm DNA. Probes were labelled by the random primer technique with [32P]deoxy-CTP to a specific radioactivity of 5–10×10^6 c.p.m./ml. Hybridization with the labelled probe was performed overnight at 65°C. After hybridization, the membrane was washed at the stringency of 0.1×SSC plus 0.1% SDS at 65°C for 15 min, and then subjected to autoradiography. The membrane was rehybridized with a human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) complementary DNA probe to normalize gene expressions. Densitometric analysis was performed using a BAS 2000 Bioimage Analyzer (Fujix, Tokyo, Japan).

Immunohistochemistry of β2-MG and CD56

Paraffin-embedded tissues sectioned at 5 µm thickness were pretreated with 0.1% trypsin for 30 min at 37°C. Sections were then treated with 0.3% hydrogen peroxide to block endogenous peroxidase and incubated with normal horse serum to reduce non-specific binding of the primary antibody. The slides were incubated with anti-β2-MG monoclonal antibody (B1G6, diluted 1:1000; Immunotech, Marseille, France), or anti-CD56 monoclonal antibody (NKH-1, diluted 1:100; Coulter Clone, Luton, UK) at 4°C overnight. Normal mouse serum was used in negative controls for specificity. For immunohistochemical staining of β2-MG, a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) was used, and the tyramide signal amplification method (Renaissance; NEN Life Science Products, Boston, MA, USA) was applied for detection of CD56 (manuscript in preparation). Counterstaining was performed with Meier’s haematoxylin.

Cell culture for in-vitro decidualization

Isolation and in-vitro decidualization of ESC were performed by the method of Kariya et al. (1991). Briefly, tissue samples were washed in RPMI-1640 medium (Gibco, Grand Island, NY, USA), finely minced and enzymatically digested with collagenase. After the digestion, most of the stromal cells were present as single cells or small aggregates, whereas most of the epithelial cells remained in larger clumps. The cell suspension was diluted twice with RPMI-1640 medium and was placed in a centrifugation tube (Corning Glass Works, Corning, NY, USA) after pipetting. The tube was allowed to remain upright for 10 min at unit gravity. The supernatant was transferred to a new tube to collect the suspended single cells. After repeating this procedure several times, the suspended cells were washed three times with RPMI-1640 and were used as ESC. Viability of at least 90% was confirmed by trypsin blue dye exclusion test. ESC (2×106 cells) were cultured in 25 cm² flasks in RPMI-1640 supplemented with 10% fetal calf serum, 100 IU/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO₂ in air. To analyse the effect of progesterone, progesterone (10⁻⁸ M) dissolved in ethanol was added to the medium after the cells became confluent, and total RNA was extracted after 9 days of culture. Ethanol alone was added for negative control. The concentration of ethanol was kept at <0.1% (v/v) to avoid growth inhibition. Culture media were changed every 3 days and stored at ~20°C until prolactin (PRL) was assayed. The PRL concentration in the culture medium was measured using the SPAC-S Prolactin Kit (Daichi Radioisotope, Tokyo, Japan) (Kariya et al., 1991). The ESC fraction contained more than 95% stromal cells, 2–3% epithelial cells and 1–2% macrophages. Contamination of endothelial cells became negligible after 3 days of culture and the ratio of epithelial cells to stromal cells decreased to <2% after 9 days of culture.

Statistical analysis

Statistical analysis of the HLA-B7 mRNA and the β2-MG mRNA levels was performed using the Mann–Whitney U test. The values of HLA-B7 and β2-MG expression represented the relative expression of these genes and G3PDH expression.

Results

mRNA expression of the HLA class I and β2-MG genes in the human endometrium

Both HLA-B7 mRNA and the β2-MG mRNA were expressed in all of the endometrial tissues examined (22 cases for HLA-B7 and 16 cases for β2-MG) at various phases of the menstrual cycle and during pregnancy (Figure 1). There was a significant correlation between expressions of the HLA-B7 gene and those of the β2-MG gene (r = 0.87, P < 0.001) (Figure 2). During the menstrual cycle, HLA-B7 gene expression level was at its highest in the late secretory phase, although the expression level in the proliferative phase was significantly higher than that during the early to mid-secretory phase (Figure 3).

We divided endometria into two groups, decidualized and non-decidualized. Decidualized endometria consist of three subgroups such as predecidual endometrium during the late secretory phase, pseudodecidual endometrium treated with EP and decidual tissue during early pregnancy. Expression of both the HLA-B7 and the β2-MG genes in decidualized endometria...
Significant correlation between the expressions of the HLA class I (HLA-B7) gene and those of the β2-MG gene in endometrial tissues ($P < 0.001$).

**Figure 3.** HLA class I (HLA-B7) mRNA expression in the endometrium during various phases of the menstrual cycle ($*P < 0.01$, $**P < 0.05$).

was significantly higher than in non-decidualized endometria (Figure 4).

**Immunohistochemical staining for β2-MG and CD56 in the human endometrium**

Immunoreactivity specific for β2-MG was detected mainly along the cell membrane and partly in the cytoplasm of positive cells. In the basal layer of the endometrium, β2-MG was not observed in either glandular epithelial cells or stromal cells. Lymphoid aggregates in the basal layer were positive for β2-MG, but these cells were few in number throughout the menstrual cycle. In the functional layer of the endometrium, immunoreactivity for β2-MG changed during the menstrual cycle and pregnancy. β2-MG was weakly positive on the epithelial cells during the proliferative phase, though appreciable staining was not observed in any part of the functional layer during the early to mid-secretory phase (Figure 5A). In the late secretory phase, however, predecidual stromal cells in the superficial layer exhibited strong immunoreactivity for β2-MG (Figure 5B). In contrast, non-decidualized stromal cells in the middle and basal layers were β2-MG-negative. In the first trimester of gestation, decidual cells showed positive staining for β2-MG, whereas glandular cells were negative. The staining intensity of decidualized cells of all three subgroups was strongly positive for β2-MG; however, non-decidualized stromal cells and glandular cells remained negative (Figure 5C).

Immunoreactivity specific for CD56 was detected mainly along the cell membrane of mononuclear inflammatory infiltrates in the endometrial stroma, and these cells were interpreted as CD56$^{bright^{+}}$ cells (Figure 5D). The number of CD56$^{bright^{+}}$ cells markedly increased during the late secretory phase, under EP treatment and in early pregnancy. CD56$^{bright^{+}}$ cells were predominantly located in the decidualized part of the endometrium, far less frequently in the spongy stratum and not in the basal layer. Accordingly, CD56$^{bright^{+}}$ cells were localized in the regions where ESC were decidualized and positive for β2-MG (Figure 5E).

**Effect of progesterone on HLA class I mRNA in cultured ESC**

Decidualization of ESC in vitro was characterized morphologically by the conversion of spindle-shaped stromal cells into epithelial-like cells with enlarged nuclei and an increased amount of cytoplasm. Decidualization of cultured ESC was also confirmed by the increase of PRL in the culture media, after 9 days of culture with the presence of progesterone (data not shown). HLA class I mRNA level in cultured ESC was markedly increased in the presence of progesterone (Figure 6).

**Discussion**

The human endometrium plays an important role in maintaining the bacteriologically sterile milieu of the endometrial lumen. In addition, aberrant immune responses have been implicated in the pathogenesis of infertility in women suffering from endometriosis (Oosterlynck et al., 1991; Vigano et al., 1991) and may be relevant to recurrent miscarriage (Lim et al., 1996). Expression of β2-MG is invariably associated with HLA class I expression (Rein et al., 1987; Seong et al., 1988). In this study, mRNA levels of the HLA class I gene in the endometrium correlated significantly with those of the β2-MG gene, and the expression of both genes was significantly
Figure 5. Immunohistochemical staining for β2-MG in the endometrium of the mid-secretory phase (A), the late secretory phase (B) and after EP treatment (C, F). Immunohistochemical staining of CD56 (D, E) in the endometrium treated with EP. A, B: bars = 40 μm, C: bar = 160 μm, D: bar = 80 μm, E, F: bars = 400 μm. Endometrial stromal cells (ESC) in the superficial layer decidualized (B) and are strongly positive but those non-decidualized (A) and ESC in the middle and basal layers (C) are negative for β2-MG. Localization of CD56bright cells (D, E) is closely associated with that of β2-MG-positive decidualized ESC (F).
The percentage of CD8+ T lymphocytes in pregnancy has been questionable (Bulmer, 1991). The presence of CD8+ T cells, and that HLA class I molecules may not be involved in their classical function of antigen presentation.

NK cells serve a unique role in the immune system regulated by a delicate balance between positive signals which initiate their effector cell function and inhibitory signals which prevent cytolysis. NK cells detect and eliminate cells without or with low levels of MHC class I molecules (Lanier and Phillips, 1996). Conversely, self HLA class I molecules have been demonstrated to protect normal cells from lysis by autologous NK cells (Ciccone et al., 1994). In the present study, CD56+ cells markedly increased in number from the late secretory phase in accordance with decidualization. In the endometrium, it is well known that a dramatic increase of leukocytes during the late secretory phase and pregnancy is accounted for by an increased number of CD56+ NK cells (Bulmer et al., 1991). Endometrial CD56+ cells exhibit MHC-non-restricted cytolytic activity in vitro (King et al., 1989; Manaseki and Searle, 1989). Pregnancy can be regarded as a kind of immune tolerance of the mother against the semi-allogeneic fetus. A non-classical HLA class I molecule, HLA-G, is expressed in the extravillous trophoblasts (McMaster et al., 1995) and can inhibit the NK activity of CD56+ cells, allowing the trophoblasts to invade into the decidual tissue from the first to the second trimester of gestation (Loke and King, 1996). In this study, the distribution of CD56+ cells correlated well with the localization of β2-MG-positive, decidualized ESC, suggesting that these cells temporally and topologically interact each other. Accordingly, induction of HLA class I molecules may be essential for decidualized ESC to escape from cytotoxicity mediated by CD56+ cells. Immunological disorder is partly attributed to the pathogenesis of endometriosis. The resistance to lysis of endometrial cells by NK-like T lymphocytes is inversely associated with expression of surface HLA class I molecules and the HLA-B7 allele inhibits the cytotoxic activity (Semino et al., 1995). It is of interest to investigate whether expression of HLA class I antigen is different between eutopic and ectopic endometria.

In conclusion, the expression of HLA class I molecules is transcriptionally activated along with the decidualization of human endometrial stromal cells. The induction of HLA class I molecules may be indispensable for the process of normal decidualization. However, it remains unknown if the expression of HLA class I molecules in decidualized stromal cells is essential for implantation and maintenance of pregnancy or not. Compared with fertile women, the endometrium from women with unexplained infertility has been reported to contain an altered subpopulation of lymphocytes (Klentzeris et al., 1994). Evaluation of endometrial HLA class I expression is necessary in patients with unexplained infertility.

**Acknowledgement**

We thank Y. Toda for his excellent assistance in immunohistochemistry. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan and by the Japanese Owners Association.
References


