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Indoleamine-dioxygenase is expressed in human decidua at the time maternal tolerance is established

U. von Rango[*], C.A. Krusche, H.M. Beier, I. Classen-Linke

Department of Anatomy and Reproductive Biology, RWTH University Aachen, Germany

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Abstract

The semi-allogeneic fetus has to be tolerated by the maternal immune system. In mice, it has been shown that inhibiting indoleamine-dioxygenase (IDO) leads to fetal rejection, suggesting a central significance for IDO in establishing maternal tolerance. Consequently, we have analyzed IDO expression in human endometrium and decidua to determine whether it may be of significance in human reproduction. Endometrial ($n=60$) and decidual ($n=68$; first and second trimester) tissue samples and isolated cells were analyzed for IDO mRNA and protein expression by real-time PCR, Western blot and immunohistochemistry. IDO expression in the decidua of proven fertile women ($n=34$) was compared to women presenting with their first pregnancy ($n=22$) and women with a history of miscarriages ($n=12$). Expression of IDO was localized in glandular epithelial cells and scattered stromal leukocytes. Expression started at the mid-luteal phase in the menstrual cycle and was high until the second trimester of pregnancy. However, glandular expression of IDO decreased during the second trimester, whereas expression in villous trophoblast started at this time. There were no significant differences in decidual IDO expression between proven fertile women and women presenting with their first pregnancy or women with a history of miscarriages. From the expression pattern we conclude that IDO may play a central role in human pregnancies for the establishment of maternal tolerance of fetal antigens. Thereby, IDO expression may be needed in each pregnancy independently from prior pregnancies, and a history of miscarriage may not reflect a general deficiency in IDO expression.

Keywords: IDO; Decidua; Human; Tolerance; First trimester; Second trimester

1. Introduction

The survival of the semi-allogeneic fetus in the mother is an immunological paradox. One attempt to explain this paradox is the concept that the fetus is not immunogenic to the maternal immune system. However, as anti-paternal antibodies are present in maternal serum, this cannot be the explanation (Beer and Billingham, 1974). In addition, trophoblast processes antigenic peptides for presentation by class I MHC molecules which are able to activate T-cells (Gobin et al., 1997; Szekeres-Bartho, 2002; Giacomelli et al., 2004). Furthermore, depending on the degree of tissue incompatibility, minor histocompatibility antigens may elicit an immunological maternal reaction leading to fetal rejection (Mellor et al., 2001).

As in mice, a reduced number of T-cells reactive against paternal antigens are present and tumor grafts bearing these antigens are accepted during pregnancy, the mother may establish specific tolerance (Tafuri et al.,

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[*] Corresponding author at: Department of Anatomy and Reproductive Biology, Medical School, RWTH Aachen, Wendlingweg 2, 52074 Aachen, Germany. Tel.: +49 241 8088927; fax: +49 241 8082508.

E-mail address: uvonrango@ukaachen.de (U. von Rango).

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1995). For this transient state of tolerance T-cells need continuous contact with their tolerogen (Tafuri et al., 1995; Alferink et al., 1995). Induction of T-cell anergy– a mechanism described for sustaining a peripheral self tolerance– by presentation of peptides from phagocytosed apoptotic cells without costimulation may also establish specific transient tolerance in the human pregnant uterus (von Rango et al., 2003a).

Another mechanism to suppress a T-cell response can be realised by the enzyme indoleamine 2,3-dioxygenase (IDO). IDO, which is induced by IFNγ in antigen-presenting cells (APC), depletes the essential amino-acid tryptophan. This causes a cessation of T-cell proliferation as T-cells exhibit a tryptophan-sensitive G1 cell-cycle arrest (Taylor et al., 1996; Munn et al., 1999; Kudo et al., 2000). This cell cycle arrest can be abrogated only by adding tryptophan in parallel with a new contact between T-cell and APC.

The significance of this mechanism for reproductive biology became evident when it was shown that, by inhibiting IDO in mice, maternal T-cell reaction against paternal antigens was induced and resulted in fetal rejection (Munn et al., 1998; Mellor et al., 2001).

This suggests that IDO may be of central importance for fetal tolerance, and interact and influence other tolerogenic mechanisms.

Recently, in humans, IFNγ-inducible IDO-expression was shown in APC (Hwu et al., 2000) and its presence in placental tissue and decidual leukocytes was described (Heikkinen et al., 2003; Kudo et al., 2001). Tolerance of fetal cells has to be established from the beginning of maternal contact with paternal antigens on extravillous trophoblast (EVT) in the first trimester. In parallel, invasion of EVT has to be limited. During the second trimester EVT reduces its invasive capacity but fetal tolerance has to be maintained until term. The regulation of this balance between tolerance and limitation of EVT may already start when endometrium is preparing for implantation and has to be adapted to the variations of the EVT invasion capacity. Therefore, we have investigated IDO mRNA and protein expression from the menstrual cycle, when endometrium is prepared for implantation, until the second trimester of pregnancy. In addition, we assessed IDO mRNA and protein expression in various isolated cell populations.

Certain complications of pregnancy, such as preeclampsia, are predominantly found in the first pregnancy and many pregnancies fail because of immunological reasons. To study whether aberrant IDO expression may be involved in such complications of pregnancy, we have compared decidual IDO expression in proven fertile women with expression in women presenting with their first pregnancy and with women with a history of miscarriage.

2. Materials and methods

2.1. Human tissue samples

Endometrial tissue was obtained throughout the menstrual cycle from normal fertile women undergoing hysterectomy due to benign uterine diseases in collaboration with the Departments of Gynaecology and Obstetrics of Marienhospital, Aachen, Luisenhospital Aachen, St. Antonius Hospital, Eschweiler, and RWTH University Aachen. Dating of each specimen was performed by menstrual history, histological examination (Noyes et al., 1959) and hormonal assessment for 17β-oestradiol, progesterone and LH on the day of hysterectomy by routine laboratory diagnostics. All patients had a regular menstrual cycle and did not receive hormones for at least 6 months before surgery. Specimens from the follicular phase (days 6–14; $n = 17$), the early luteal phase (days 15–19; $n = 14$), the mid-luteal phase (days 20–24; $n = 13$) and the late luteal phase (days 21–28; $n = 16$) were included.

Decidual tissues were collected from legal termination of normal healthy pregnancies in collaboration with the Casa Maastricht, the Netherlands. Informed consent of patients was obtained. Patients included in this study were proven to be pregnant by hCG-serum test and sonographic control immediately before surgery.

Gestational age was determined by the duration of amenorrhoea, analysis of individual cycle data and by the caput-rump length (CRL) determined sonographically. Termination was obtained by vacuum aspiration. Fragments of decidua basalis (with trophoblast) and parietalis (without trophoblast) were taken. Specimens were collected from first and second trimester of pregnancy (5–12 and 13–17 weeks after the last menstrual period) and were identified as decidua parietalis and decidua basalis by histological examination and cytokeratin staining of the trophoblast,as described before (von Rango et al., 2001). Proven fertile women (without any spontaneous abortion and at least one child; weeks 5–6, $n = 6$; weeks 7–8, $n = 8$; weeks 9–10, $n = 6$; weeks 11–12, $n = 4$; weeks 13–14, $n = 5$; weeks 15–16, $n = 5$; aged from 21 to 42 years; mean, 31 years) who had not received any exogenous hormones within the last 3 months prior to pregnancy were included in this study in comparison to women presenting with their first pregnancy (weeks 5–6, $n = 3$; weeks 7–8, $n = 4$; weeks 9–10, $n = 4$; week 11–12, $n = 2$; weeks 13–14, $n = 4$; weeks 15–16, $n = 5$;