Evaluation of oestrogen and progesterone receptor expression in uterine mucosal lymphocytes

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Expression of the oestrogen and progesterone receptors on uterine mucosal leukocytes has been examined by dual immunohistology. Neither the oestrogen receptor nor the progesterone receptor was expressed by lymphocytes, macrophages or the distinctive population of uterine natural killer (NK) cells. Although the accumulation and survival of these NK cells appears to be hormonally dependent, the effects must therefore be indirect.

Key words: human endometrium/NK cells/oestrogen receptor/progesterone receptor/uterine leukocytes

Introduction

During early pregnancy the uterine mucosa, the decidua, is infiltrated by large numbers of natural killer (NK) cells with a distinctive phenotype, CD56high CD16- mCD3- (King et al., 1991; Loke and King, 1995). These uterine NK cells are also present in the non-pregnant endometrium where they increase in number from the proliferative to the secretory phase (King et al., 1989; Bulmer et al., 1991). The change in numbers of uterine NK cells during the menstrual cycle suggests that these cells are under hormonal regulation, in particular oestrogen and progesterone, but the mechanism of action of these hormones is not known. It has not been established whether these uterine NK cells in both non-pregnant endometrium and decidua express oestrogen receptors (ER) or progesterone receptors (PR), although numerous immunohistological reports have described the distribution of ER and PR in the stromal cells of the mucosa (Garcia et al., 1988; Lessey et al., 1988; Press et al., 1988; Snijders et al., 1992; Wang et al., 1992; Bergqvist et al., 1993; Critchley et al., 1993; Wu et al., 1993; Amso et al., 1994). The uterine NK cells are the predominant leukocyte population in first trimester decidua, accounting for ~70% of all CD45+ bone marrow-derived (B) cells. Other leukocytes present are CD14+ macrophages (~20%) and CD3+ T cells (~10–20%). B cells are scarce and are only found in the basal lymphoid aggregates. The immunolocalization of ER and PR is optimal on formalin-fixed sections. However, the CD56 antigen is destroyed by this procedure, although, in contrast, the CD45 antigen survives formalin fixation and paraffin embedding.

Therefore we have used dual-colour immunohistology to determine if all CD45+ uterine leukocytes (mainly comprising CD56+ cells) express ER or PR.

Materials and methods

Tissues

Tissue samples were taken from pregnant hysterectomy specimens and routine vaginal terminations of pregnancy, as described previously (King et al., 1991). The gestational age ranged from 6 to 12 weeks. Samples of non-pregnant endometrium were obtained from hysterectomy specimens as before (King et al., 1989). These were dated by histological criteria and included samples taken from throughout the menstrual cycle. In all, 18 samples were examined of proliferative (n = 3) and secretory endometrium (n = 3), decidua basalis (n = 6), and parietalis (n = 3) and placental villi (n = 3). The tissue was fixed in neutral buffered formalin and routinely processed into paraffin blocks.

Immunohistology

All paraffin sections were dewaxed in xylene and rehydrated through gradations of ethanol to water. Sections were then boiled in 0.01 M sodium citrate buffer, pH 6.0, for 1 min at pressure inside a standard pressure cooker to unmask antigens. All incubations were performed in a humid atmosphere at room temperature, and sections were washed twice for 5 min in phosphate-buffered saline (PBS) between steps, unless otherwise indicated. Secondary antibody was pre-absorbed with human AB serum (10%; Sigma, Poole, Dorset, UK; 30 min at room temperature) and centrifuged before use. After washing in PBS, the sections were blocked with normal horse serum (Vector Laboratories, Peterborough, Cambs, UK; 1/50 in PBS, 20 min). Excess serum was blotted from the edge of the section before the application of mouse monoclonal antibodies to PR (NCL-PGR), ER (NCL-EH-LH2) (both Novocastra Laboratories, Europath Ltd, Bude, Cornwall, UK; 1/30 in PBS) and cytokeratin (MNF 116, Dako Ltd, High Wycombe, Bucks, UK; 1/200 in PBS for 30 min). Primary antibody was detected using the standard Vectastain ABC technique (Vector), which employs the use of biotinylated horse anti-mouse immunoglobulin G secondary antibody (1/200 in PBS, 30 min) followed by avidin–horseradish peroxidase complex (ABC; 30 min). Bound peroxidase enzyme was developed with diaminobenzidine (Sigma; 0.5 mg/ml in PBS + 0.03% H2O2) to give an insoluble brown precipitate.

Sections were then double labelled to visualize leukocytes using the alkaline phosphatase—anti-alkaline phosphatase (APAAP) technique. Washing steps were performed as before, but Tris-buffered saline (TBS) was substituted for PBS before the application of mouse APAAP. Monoclonal antibody Leu12 (Becton Dickinson Ltd, Oxford, UK; 1/50 in PBS), which recognizes B cells (CD19), was used as an irrelevant control antibody because scarce B cells are found in the uterine mucosa and these are only present in the basal lymphoid aggregates. Sections were blocked with normal rabbit serum (Vector,
1/50 in PBS, 20 min) before application of the monoclonal antibody to leukocyte common antigen (CD45; Dako; 1/50 in PBS, 30 min). Intermediate rabbit anti-mouse immunoglobulins (Dako; 1/20 in PBS, 30 min) were applied to link primary antibody to the final step, mouse APAAP (Sigma; 1/40 in TBS, 30 min). Bound alkaline phosphatase was developed with Texas Red (Sigma; Fast Red TR AS-MX substrate tablets in TBS). Sections were washed briefly in TBS, counterstained for 2 min with Carazzi haematoxylin to give a pale nuclear stain, and mounted with glycerol/gelatin (Sigma).

Results

Non-pregnant endometrial tissue sections exhibited positive nuclear labelling of both glandular epithelium and stromal cells with both monoclonal antibodies to PR and ER. The intensity of labelling and the proportion of positively labelled cells were variable. For example, both positive and negative cells were visible within some glands, whilst others were either all positive or all negative. In proliferative phase endometrium, glands which were closest to the myometrium were strongly positive for PR, whilst those closer to the lumen contained a higher proportion of negative cells. Most glands stained positively for ER with few negative individual cells (Figure 1A). This pattern of labelling within the glands changed in the early secretory phase, with ER labelling first becoming weaker. Very few epithelial cells expressed either receptor by the mid-secretory phase, and labelling was only very weak or negative in the late secretory phase. Stromal cells displayed positive labelling for both receptors, although ER labelling was weaker and more variable. By the late secretory phase, PR staining of the stromal cells was still seen but was very weak or negative for ER (Figure 1B). A high proportion of myometrial smooth muscle cells were strongly positive for PR and less so for ER. Endometrial cells lining vessels in both the endometrium and the myometrium were negative. Endometrial leukocytes identifiable by red CD45+ surface labelling displayed no nuclear labelling with either PR or ER in any of the endometrial samples studied (Figure 1A and B).

In the pregnant uterus, decidual stromal cells continued to express varying concentrations of PR but no staining was seen for ER. Labelling was generally stronger in those cells closest to the myometrium, with a smaller proportion of positive cells in the decidua compacta. PR was expressed very strongly in the majority of myometrial smooth muscle cells, but not by glandular epithelium. Staining for ER was not found in the pregnant uterus, where infiltrating trophoblast cells were identified by cytokeratin staining of serial sections (data not shown). Endothelial cells, leukocytes and all populations of trophoblast (syncytiotrophoblast, columns, extravillous, intra-vascular, placental bed giant cells) did not express PR (Figure 1C and D). Therefore, in the decidua only stromal cells express PR.

Discussion

Our results show that CD45+ uterine leukocytes, including the main population of CD56+ NK cells and the smaller numbers of macrophages and T cells, in both the endometrium and the decidua do not express ER or PR. Although immunohistology is a relatively insensitive technique, nuclear staining for ER and PR was detected in other cell populations in the uterus, indicating that if these hormone receptors are present, then they are present at very low concentrations on leukocytes. Therefore, although these hormones appear to influence the recruitment of these CD56+ cells in vivo, their effects must be acting indirectly.

The proliferation of CD56+ cells occurs in the uterus in vivo, commencing immediately post-ovulation and continuing in the early decidua (Pace et al., 1989; King et al., 1991). If pregnancy does not occur, uterine NK cells undergo apoptosis premenstrually at the time that progesterone concentrations are rapidly falling (King et al., 1989). This apoptosis of uterine NK cells gave rise to the misnomer 'endometrial granulocytes', and occurs before there are any other visible features of menstruation. In artificial cycles in women with premature ovarian failure, lymphocyte numbers are increased greatly after progesterone treatment (Booker et al., 1994), and the numbers of CD56+ cells are very high in women taking exogenous progesterone for menstrual disorders (unpublished results). It seems that in these situations progesterone is not acting directly on the NK cells, but may instead exert its effect through stromal cells which are the only cells in the uterine mucosa that continue to express PR in the late luteal phase and in early pregnancy.

There is a close physical association between CD56+ cells and stromal cells. Binding of these uterine lymphocytes to monolayers of stromal cells has been observed, and is dependent on the expression of a variety of β1 integrins (Burrows et al., 1993, 1995). In addition, we have found that uterine CD3+ lymphocytes will proliferate in the presence of a low dose of interleukin (IL)-2 when co-cultured with irradiated stromal cells (unpublished results). The observation that bcl-2 (a proto-oncogene responsible for the suppression of apoptosis) is expressed in uterine CD56+ cells in the luteal phase (Koh et al., 1995) and is also strongly expressed in decidual CD56+ cells (unpublished results) suggests that stromal cells may influence both survival and proliferation. Stromal cell differentiation occurs under the influence of oestrogen and progesterone. This process commences before implantation in humans (pre-decidual change), and involves the secretion of both soluble products and extracellular matrix components which appear essential for uterine CD56+ NK cell survival and proliferation (Loke and King, 1995). It is not yet clear which decidual products may influence NK cell proliferation in vivo. In vitro, IL-2 is the only substance so far identified that is capable of stimulating such proliferation (Nishikawa et al., 1991; King et al., 1992; Saito et al., 1993a). As IL-2 also activates decidual NK cells to become potent lymphokine-activated killer cells capable of killing trophoblast (King and Loke, 1990), it seems unlikely that IL-2 would provide the proliferative stimulus in vivo. Furthermore, IL-2 has not been detected in first trimester decidua in normal pregnancies (Saito et al., 1993b; Jokhi, 1994).
ER and PR expression in uterine mucosal lymphocytes

Figure 1. (A) A section of normal mid-proliferative phase endometrium. There is abundant oestrogen receptor nuclear staining (brown) of both glandular cells and the majority of stromal cells. Two CD45+ cells (red) show no brown nuclear reactivity (magnification ×40). (B) A section of secretory endometrium (post-ovulation days 8—9). Progesterone receptor (PR) nuclear staining of some stromal cells is seen (brown). Numerous CD45+ cells (red) exhibit no staining for PR (magnification ×40). (C) A section of decidua at 8 weeks of gestation. The PR staining (brown) of stromal cell nuclei is not as strong as in secretory endometrium. CD45+ leukocytes (red) are negative for PR (magnification ×40). (D) A section of decidua at 8 weeks of gestation showing a small arteriole. The endothelial cells are not staining for PR (brown), although surrounding stromal cells show positive nuclear reactivity (magnification ×40).

terone for their survival and proliferation in vivo. However, our demonstration of the lack of an appropriate ER or PR on uterine lymphocytes indicates that these hormones are acting indirectly via other cells in the stroma, presumably on stromal cells themselves, because macrophages also do not express ER or PR. A uterine mucosal stromal cell product that might be considered is prolactin (Handwerger and Brar, 1992). The prolactin receptor belongs to the same family of cytokine
receptors as IL-2 (Sato and Miyajima, 1994). It is not known which cells in the decidua and implantation site express this receptor; however, we were unable to detect the proliferation of decidual lymphocytes with soluble prolactin (unpublished results). Other candidates include members of the interleukin family, particularly IL-15 whose receptor shares the β-chain with the IL-2 receptor (Taga and Kishimoto, 1995).

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