Endometrial receptivity: expression of α3β1, α4β1 and αVβ1 integrins in women with impaired fertility

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SUMMARY

Advances in immunohistochemical methods with the specificity of poly- and monoclonal antibodies allow the description of the endometrial receptivity, which is characterized by the ability of secretion of phase specific proteins and glikoproteins by epithelial and stromal cells. We studied the differences in the expression of α3β1, α4β1 and αVβ1 integrins in endometrium of women with recurrent miscarriages and women with unexplained infertility. The endometrial tissue was collected during hysteroscopy performed between 7th and 9th day after ovulation. The immunohistochemical evaluation of α3β1, α4β1 and αVβ1 integrin expression was determined in all endometrial biopsies. Staining intensity of α3β1 in glandular epithelium and endometrial stroma was similar in both groups. In women with recurrent miscarriages we noted a lower concentrations of the α4β1 and αVβ1 integrins during the midluteal phase than in women with unexplained infertility. Moreover, integrins α4β1 and αVβ1 were expressed more frequently in glandular epithelium and endometrial stroma of women with unexplained infertility than those of women with recurrent miscarriages. However, αVβ1 staining in endometrial stroma was stronger than that of α4β1. It can be concluded, that these integrins may play an important role in the implantation process. Reproductive Biology 2001, 1(2): 85-94

Key words: endometrial receptivity, integrins, progesterone, recurrent miscarriages, unexplained infertility

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INTRODUCTION

Research concerning endometrial receptivity has been recently intensified. Assuming that the molecular status of the endometrium has a direct influence upon implantation, which is far greater than that of a level of progesterone and of other markers of the luteal phase, we hope that advances in this field could give better insight into the mechanisms and causes of recurrent miscarriages and, ultimately, into treating this condition. The most common method for evaluating the luteal phase dysfunction in states of impaired fertility, including recurrent miscarriages, is endometrial biopsy and histological evaluation according to morphological guidelines proposed by Noyes and Hertig [13] in 1950. This classification enables the detection of the cyclical changes occurring in endometrium.

Advances in immunohistochemical methods with specificity of poli- and monoclonal antibodies allow the identification of the localization of different factors, which are used to describe endometrial receptivity such as: estrogen and progesterone receptors, leukemia inhibiting factor (LIF), interleukin-1, macrophage colony stimulating factor, cell proliferation marker (K167) and many adhesion molecules, including kadherins and integrins [16].

Integrins are a major class of cell adhesion molecules. These interesting glycoproteins are cell surface heterodimers formed by the association of one of 16 α subunits and one of at least nine different β subunits. The combinatorial potential of the system is much larger than the currently described integrins and reflects the constraints of preferred pairing of particular subunits. These pairings determine ligand specificity, which may also be modified by cell type and membrane composition [19].

It has been suggested that the expression of α1β1, α4β1, and αVβ3 integrins by the glandular epithelium could serve as a marker for endometrial receptivity during the "implantation window" [9]. However, the successful development of pregnancy depends on interactions between different integrin molecules and their selective binding. Endometrial receptivity is not only a function of single receptor-ligand complex; it should be considered as a diverse expression of various adhesion molecules with different ligand binding capacities and signal transduction properties [15, 16]. That is why immunohistochemical assessment of endometrial integrin expression can provide more information than typical histological assessment. Histochemical analysis will not replace histological evaluation of endometrium, but it can certainly reveal functional deviations in otherwise normal endometrium [12].

We decided to study differences in the expression of integrins between women with recurrent miscarriages and women with unexplained infertility based on the two following facts: 1/ expression of α3β1 integrin increases during the late luteal phase and early pregnancy, and it is connected with decidualization of the stroma; 2/ α4β1 integrin is expressed on the surface of epithelial cells during the implantation window, i.e. between 6th and 10th day after ovulation [19]. In this study we describe the expression of α3β1, α4β1 and αVβ1 integrins in midluteal endometrial tissues of women with unexplained infertility and recurrent miscarriages.
MATERIALS AND METHODS

Materials

Endometria were collected from 22 women. Group No 1 comprised endometria of 11 women with recurrent miscarriages and group No 2 consisted of 11 infertile women. The Ethics Committee of the Karol Marcinkowski University of Medical Sciences in Poznań approved the research protocol. The age of the women with recurrent miscarriages was between 21 and 32 years (mean 30.6 years old) and the number of miscarriages between two and five. The infertile women were between 26 and 37 years old (mean 32 years old) and the infertility period lasted from 1.5 to 7 years (mean 4.2 years).

The endometrial tissue was collected during hysteroscopy performed during the second phase of the cycle, between 7th and 9th day after ovulation assessed by ultrasonography. In each patient early pregnancy was excluded by βhCG serum concentration measurement. Endometrial dating correlated well with the date of ovulation assessed by repeated vaginal sonography. Follicular rupture was considered to have occurred when the diameter of the dominant follicle was decreased by more than 3 mm and free liquid was observed in the cul-de-sac.

Methods

The expression of α3β1, α4β1 and αVβ1 integrins was evaluated in the endometrium. Immediately after biopsy the material was frozen and cut on a cryostat for staining. Four μm sections were cut and mounted on slides. The sections were fixed in acetone at 21°C for 10 minutes, and washed in H2O2 for 10 min followed by TRIS buffered saline (TBS). After drying, the sections were incubated with appropriate dilutions of monoclonal primary and secondary mouse antibody to human α3β1 or α4β1 or αVβ1 integrin for 12 hours in 5°C. Slides were washed twice in TBS 3-3-diaminobenzidine. Hydrogen peroxide was used for the visualization of integrin:antibody complex. Both primary and secondary antibodies were supplied by DAKO (EnVision system), Denmark.

The staining intensity of endometrial components was evaluated by a semi-quantitative scoring system as follows: absent –; week or focal +; moderate ++; and strong ++++. Negative control was done on slides incubated without primary antibody, and positive control on samples with known integrin expression.

On the day of endometrial biopsy in women with recurrent miscarriages blood samples were drawn for the evaluation of progesterone concentrations. After centrifuging, the plasma was frozen in –20°C, and left for further steroid analysis. The concentration of progesterone was evaluated by electrochemiluminescence (ECL) assay using the PROGESTERONE II KIT from Roche. It has the range from 1.02 – 53.0 ng/ml and sensitivity threshold of 0.03 ng/ml. All evaluations were performed on ELECSIS 1010 manufactured by Roche.
RESULTS

I. Staining intensity of $\alpha_3\beta_1$, $\alpha_4\beta_1$ and $\alpha\nu\beta_1$ integrins in endometrial epithelial cells and stroma in women with recurrent miscarriages

The epithelial $\alpha_3\beta_1$ was the most prominent integrin among all integrins studied in endometrial biopsies collected during the midluteal phase of the cycle from women with recurrent miscarriages. Its staining was noticeable in nine out of 11 specimens and in six of those the reaction was described as strong. There was no reaction for $\alpha_3\beta_1$ integrin in glandular epithelium of two biopsies. The staining of $\alpha_3\beta_1$ in the stroma was observed in three out of 11 specimens and its intensity was described as weak (table 1).

The staining intensity of $\alpha_4\beta_1$ integrin in glandular epithelium was weaker than that of $\alpha_3\beta_1$ and it was discovered only in five out of 11 specimens; the reaction was described as moderate in two and as weak in the remaining three patients. The reaction for $\alpha_4\beta_1$ integrin in the stroma was noted in three biopsies and it was depicted as weak (table 1; fig 1).

<table>
<thead>
<tr>
<th>Patient's number</th>
<th>$\alpha_3\beta_1$</th>
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<td>stroma</td>
<td>glandular epithelium</td>
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Tab. 1. Staining intensity of integrins in glandular epithelium and stroma of women with recurrent miscarriage in the midluteal endometrial biopsies
Different observation was made in case of $\alpha V\beta 1$ integrin which was absent in glandular epithelium. This integrin was present in five out of 11 biopsies of stroma; the reaction was described as weak, moderate or strong in one, two and two patients, respectively (table 1).

The progesterone concentration in plasma was analyzed in association with the immunoreaction of $\alpha 4\beta 1$ assuming that both subunits are progesterone dependent. It is of interest that in patients with low plasma P4 concentrations (<10 ng/ml) no staining of $\alpha 4\beta 1$ integrin in both glandular epithelium and stroma of endometrium was found.

II. Staining intensity of $\alpha 3\beta 1$, $\alpha 4\beta 1$ and $\alpha V\beta 1$ integrins in epithelial cells and stroma in infertile women

Staining intensity of integrins in endometrium in women with unexplained infertility differed from the expression of those integrins in women with recurrent miscarriages. The differences were observed between all three integrins studied, however the most prominent one concerned the $\alpha V\beta 1$ integrin (tables 1 and 2).
**Tab. 2.** Staining intensity of integrins in glandular epithelium and stroma of women with unexplained infertility in the midluteal endometrial biopsies

<table>
<thead>
<tr>
<th>Patient's number</th>
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<th>α3β1 stroma</th>
<th>α4β1 glandular epithelium</th>
<th>α4β1 stroma</th>
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In women with unexplained infertility the reaction for αVβ1 integrin was very distinct both in epithelium and stroma. Expression of αVβ1 integrin was not observed only in four patients from this group, while in the other group epithelial αVβ1 integrin was not found in any of our patients. The staining intensity of αVβ1 integrin in endometrial stroma was also higher than in the first group; it was found in ten out of 11 patients, and in the majority of cases the reaction was described as moderate and strong. In group No 1 staining of αVβ1 integrin in endometrial stroma was noted in five out of ten patients.

There were also differences in staining intensity of α4β1 integrin in women with recurrent miscarriages and unexplained infertility. In the latter group immunoreaction of α4β1 was more pronounced, especially in endometrial epithelium. We observed the presence of this integrin in eight out of 11 patients, while in group No 1 it was present only in five out of 11 women. The slightest difference was found when examining the α3β1 integrin, both in endometrial epithelium and stroma. In group No 2 staining for epithelial α3β1 integrin was observed in all specimens, while in group No 1 in nine out of 11 specimens. Immunohistochemical reaction of stromal α3β1 integrin was similar in both groups (fig 2).
**DISCUSSION**

Endometrial receptivity, according to Yoshinaga [18], is characterized by: structural and functional transformation of apical surfaces of epithelial cells, secretion of phase specific proteins and glikoproteins by epithelial cells, the ability of stromal cells to differentiate in response to certain signals as well as structural reorganization of basal membrane and extracellular matrix. Specific mechanisms guiding endometrial receptivity still remain unidentified. Finding a specific marker of endometrial receptivity for blastocyst implantation could lead to better treatment of women with impaired fertility. Adhesion molecules, especially integrins, are beginning to prove to be very promising receptivity marker [11].

Lessey et al. [10, 11] and other authors [16] showed that integrins are expressed by epithelial cells in a phase dependant manner. While the αVβ1 integrin is present during menses, the α1, α4, β3 subunits remain repressed in the proliferative phase. Progesterone dependent α1 and β1 subunits are expressed during ovulation and secretory phase. Similar to α1 subunit, α4 subunit is also progesterone dependent, but its expression is turned off 10 days after LH peak. Women with luteal phase deficiency lack α1 and β1 subunits [10].
AlfaVβ3 is the phase specific integrin: αV subunit is secreted by glandular epithelium throughout the whole cycle, while β3 subunit appears on 20th day of the cycle. However, Beliard et al [1] did not confirm the cyclic changes of α1 subunit expression in stromal cells, while Hu and Rogers [5] did not validate epithelial αVβ3 changes during the menstrual cycle. On the other hand it has been suggested that the α6β4 integrin is a cycle dependent endometrial molecule [7].

The subject of our research were endometria sampled in the midluteal phase during the so-called implantation window in women with recurrent miscarriages and unexplained infertility. In women with recurrent miscarriages, epithelial α3β1 integrin was the most prominent one, while epithelial α4β1 was much weaker (tables 1 and 2). We found that the expression of integrins in endometrium in women with recurrent miscarriages differed markedly from those of women with unexplained infertility.

Unfortunately, it was not possible in the current study to examine endometria of normal fertile women. However, Gonzalez et al. [3] by means of flow cytometric analysis have investigated concentrations of α1, α4, αV and β3 integrin subunits in endometrial stromal and epithelial cells in two groups of women throughout the menstrual cycle: normal fertile women and women with unexplained infertility. The epithelial α4 integrin subunit (α4β1, a fibronectin receptor) had a low expression in fertile and infertile women, throughout the menstrual cycle.

In our material, the expression of epithelial α4 integrin subunit in the midluteal phase was higher than the stromal subunit and stronger in women with unexplained infertility. A moderate expression of the stromal α4 integrin in infertile women during different phases of the menstrual cycle has been described earlier [13, 17]. Klentzeris et al [6] reported that the α4 expression is frequently absent in patients with unexplained infertility.

We partially concurred with the above data because we observed in the majority of women with unexplained infertility a very apparent expression of epithelial α4β1 integrin, while, similarly to the above mentioned authors, we noted a weak expression of stromal α4β3 integrin in half of our patients. The latest data from Lassey [9, 11] suggest that the lack of α4 expression occurs sporadically in many women, and it is not an indication of pathology. It is also possible that the differences in methodology or choice of antibody as well as integrin ligand binding capabilities may account for such discrepancies. Honda et al [4] found that ligand binding may alter the conformation of some integrins leading to the inhibition of the binding of some antibodies. Beta3 integrin subunit seems to have a bigger impact, and it has been proposed as a more reliable biochemical marker of the failure of endometrial receptivity.

Gonzalez et al [3] confirmed that the expression of epithelial β3 subunit was significantly higher in the fertile women at the time of the implantation window. Infertile women expressed lower epithelial β3 during the mid-secretory phase.
Defects in epithelial β3 expression have been postulated by Lassey et al [9] as a cause of infertility. Also Creus et al. [2] found that almost 50% of endometria from infertile patients did not express the αVβ3 integrin in endometrial glands during the window of implantation. There were no differences in the pattern of endometrial integrin expression during the implantation window between women becoming spontaneously pregnant within one year after the study and those patients who did not get pregnant [2]. Our results differ slightly from the above data. We observed expression of epithelial αVβ1 integrin in 64% and expression of stromal αVβ1 integrin in 91% of samples collected from patients with unexplained infertility during the implantation window. In patients with recurrent miscarriages αVβ1 immunoreaction was seen only in endometrial stroma and in less than half (5/11) of the cases. In our opinion the differences in expression of integrins observed by us during the implantation window in endometrium from women with recurrent miscarriages and unexplained infertility confirms that these molecules play an important role in the implantation process.

It can be assumed that merely analyzing the expression of certain integrins will not give the final answer as to what is the defect in endometrium in women with recurrent miscarriages, even more so that most of this studies are conducted in nonconceptional cycles. We should also study mechanisms that govern the expression of integrins in the endometrium that is: steroid hormones, cytokines and growth factors.

It has been postulated that progesterone or the ratio of estradiol to progesterone could control expression of integrins [17]. However some authors found comparable concentrations of progesterone, independently whether endometrium were in or out of phase or if it expressed αVβ3 integrin or not [2].

On the other hand we observed a dependence of progesterone concentrations measured on the day of the endometrial biopsy on the expression of α4β1 integrin. In those women whose endometrium failed to express stromal and epithelial α4β1 integrin, the progesterone concentration was low (<10ng/ml). A small number of samples does not justify the thesis that a low concentration of progesterone could be a marker for impaired receptivity of the endometrium, even more so in view of the fact that one of these women, three months after completing the study, became pregnant and later delivered a healthy baby.

In conclusion, some differences have been found in the endometrial expression of α4β1 and αVβ1 integrins during the midluteal phase between women with recurrent miscarriages and with unexplained infertility. The staining intensity of α3β1 integrin in glandular epithelium and endometrial stroma was similar in both groups. It is of interest that the presence of α3β1 integrin in glandular epithelium was observed more frequently in comparison to the other integrins. It is possible that this direction of research will help us in the future to define the reasons for recurrent miscarriages in women, of whom 50% still remain undiagnosed.
REFERENCES


