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Aberrant positioning of trophoblast and lymphocytes in the feto-maternal interface with pre-eclampsia

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Abstract Pregnancy represents the growth of an allograft where fetal trophoblast cells evade immune rejection and invade maternal tissue. There should be a balance between fetal trophoblast and maternal immune-responsive cells and alterations in the proportion of these cells may relate to pregnancy disorders. To test this, the decidual tissue of placental bed biopsies was examined and trophoblast cells and lymphocytes were quantified morphometrically; spiral arteries were classified as unchanged, transformed or affected by acute atherosis. Normal pregnancy (*n*=19) was characterized by the transformation of about one half of all spiral arteries within the placental bed. We found that 40% of all lymphocytes were $CD56^+$ uterine NK cells and 60%, $CD3^+$ T-lymphocytes; about 30% of these were $CD8^+$ T cells. Intrauterine growth retardation in the context of preeclampsia (*n*=15) was accompanied by reduced trophoblast numbers within smaller and more tortuous arteries and an increase in the proportion of $CD56^+$ uterine NK cells and $CD8^+$ T lymphocytes in the decidua (70% of all $CD3^+$ cells). In the case of pre-eclampsia without fetal growth retardation (*n*=14) no increase in $CD56^+$ uterine NK cells was seen, while $CD8^+$ T lymphocytes were significantly increased compared with the normal level (50% of all $CD3^+$ cells). Fetal growth retardation is associated with poor transformation of spiral arteries and characterized by an increase of uterine NK cells. Symptoms of pre-eclampsia are independently associated with an increase in the cytotoxic T subset of decidual lymphocytes. Pre-eclampsia and related fetal growth retardation are seemingly caused by an enhancement of the maternal cytotoxic defence against the fetal allograft.

Key words Pregnancy · Placenta · Pre-eclampsia · Lymphocyte · NK cell

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Introduction

During pregnancy, the specialized tissues of mother and fetus interact in a specific way. The uterus of a mother must undergo rapid adaptive changes as it comes in contact with the placenta and the membranes containing the fetus. Between these organs, there is the feto-maternal interface-often called the decidua-which is infiltrated by fetal trophoblast cells [7, 16] and by maternal immune cells. Beside macrophages and T lymphocytes, the feto-maternal interphase is populated by uterine NK cells, whose function is largely unknown [5, 12, 15, 17]. One main outcome of trophoblast invasion is the transformation of maternal spiral arteries, the purpose of this being to supply more blood to the fetus. The destruction of muscle layers of the blood vessels and the replacment by an undefined matrix has been described several times and found to depend on the invasive action of the trophoblast cells [4, 9, 10]. However, the large number of maternal macrophages and lymphocytes in the surrounding tissues has not been commented on extensively.

A biopsy technique to obtain tissue from the feto-maternal interface was introduced in 1958 [2, 11]. The so-called placental bed biopsy is excised from the inside of the uterus during a caesarean section and includes parts of the myometrium. In contrast, the biopsy material used in this study was obtained with a curette; it was larger in quantity but its spatial orientation was lost, and the strategy of the examination therefore had to be modified.

Pre-eclampsia and the related fetal growth retardation are interpreted as consequences of defective trophoblastic invasion [4, 9]. This study examined aberrant patterns of trophoblast invasion in the context of possible alterations in the lymphocyte infiltration of the placental bed,

with special reference to the topograpy of the feto-maternal interface. Maternal blood vessels were evaluated morphometrically, and the extent and distribution of fetal trophoblast cells and the infiltration by maternal lymphocytes were quantified using immunohistochemically stained serial sections.

Materials and methods

The experimental protocol was approved by the university ethical committee. Informed consent was obtained from all the mothers, and a placental bed biopsy was performed during each of more than 50 caesarean sections done in the University Hospital of Zürich. Immediately after delivery of the placenta, tissue was removed from the previously marked site of the placental bed by means of a large curette. Although no incisions were made into the deeper layers of the uterine wall, fragments of the myometrium were usually contained in the biopsy material. About one quarter of the material was snap-frozen in liquid nitrogen, while the rest was fixed in formalin.

Caesarean section was performed either as an emergency intervention because of severe fetal distress or for obstetrical reasons in a planned way; the latter group served as controls (n=19). The mean gestational age in the group of controls was 36 weeks. Seven women had established labour with regular contractions and the cervix had already opened more than 4 cm; in most of these cases surgical intervention was neces-sary because of a small pelvis and antecedent caesarean section. Twelve women had no signs of uterine contraction at the time of caesarean section, and in most of these the section was performed because of breech presentation or antecedent caesarean section.

The disease states requiring surgery were defined as follows: a diagnosis of intrauterine growth retardation was made if during the course of pregnancy fetal growth had declined and fallen under the 5th percentile on ultrasound examination, compared with the percentile curves based on a survey of the local population. Growth had to be classified as asymmetrical, and malformations or chromosomal abnormalities should not be present.

The diagnosis of pre-eclampsia required hypertension and proteinuria in the mother as minimum clinical criteria (n=15). Diastolic blood pressure had to exceed 90 mmHg during pregnancy with normal blood pressure previously in the nonpregnant state; proteinuria had to be at least 0.5 g/l within 24 h. Cases of pre-eclampsia with fetal growth retardation were grouped separately. In our series, this was always associated with a small placenta exhibiting signs of circulatory disturbance (n=14). The mean gestational age in both these groups of women with pre-eclamptic pregnancies was 33 weeks.

For immunohistochemistry, formalin-fixed tissue was embedded in paraffin and 5-µm-thick serial sections were cut from one block per case. One slide was stained with haematoxylin-eosin, and the next slide was stained immunohistochemically with Lu5 (BMA, dilution 1:250). The cytokeratin antibody Lu5 marks the epithelial lining of maternal endometrial glands (internal positive control) (Fig. 3) and stains fetal trophoblast cells very strongly [3, 7, 16]. Snap-frozen tissue was used for immunohistochemical detection of T lymphocyte surface markers CD3 (Dako M756; dilution 1:200), CD8 (Dako M707; 1:40) and CD4 (Dako M0716; 1:20) and the natural killer cell marker CD56 (Becton Dickinson 347740; dilution 1:10) in serial sections (Fig. 4). The immunohistochemical staining was performed with standard PAP and APAAP protocols. The counts of $CD4^+$ cells had to be corrected by the deduction of $CD68^+$ macrophages (Dako PG-M0876; 1:10) which also express CD4. Immunhistochemical staining of B lymphocytes was always done concomitantly with CD 19 (Dako M740;1:80) but revealed only single cells in decidual tissue.

The dimensions of the spiral arteries and the densities of various cells in the placental bed were measured in sections cut from the block with the most representative tissue; evaluation was done without knowledge of the clinical diagnosis. Measurements were taken by computer-assisted morphometry on a microscope equipped with a video camera (Zeiss AxioHOME).

The maximal interior transverse diameter of the lumen of all cross sections of all spiral arteries within one block of each biopsy was measured. Owing to the coiling of the "spiral" arteries there are always several cross sections of the same artery lying close to each other; this feature helps to exclude other, so-called basal, arteries from the evaluation. Veins could be excluded from the morphometric analysis since their thin walls and usually slit-like lumens were readily recognizable. The orientation of a cut through an artery has a great influence on the magnitude of the longitudinal diameter; the transverse diameter was chosen because it is less affected.

Trophoblast cells and lymphocytes were counted in the wall and in the vicinity of two sections of each type of spiral artery and within 2.5 mm^2 of pure interstitial tissue in each case. Cells were counted in immunohistochemically stained sections; densities were calculated as cells per square millimetre. The level of significance of the differences obtained was determined by the use of the nonparametric Wilcoxon test.

Results

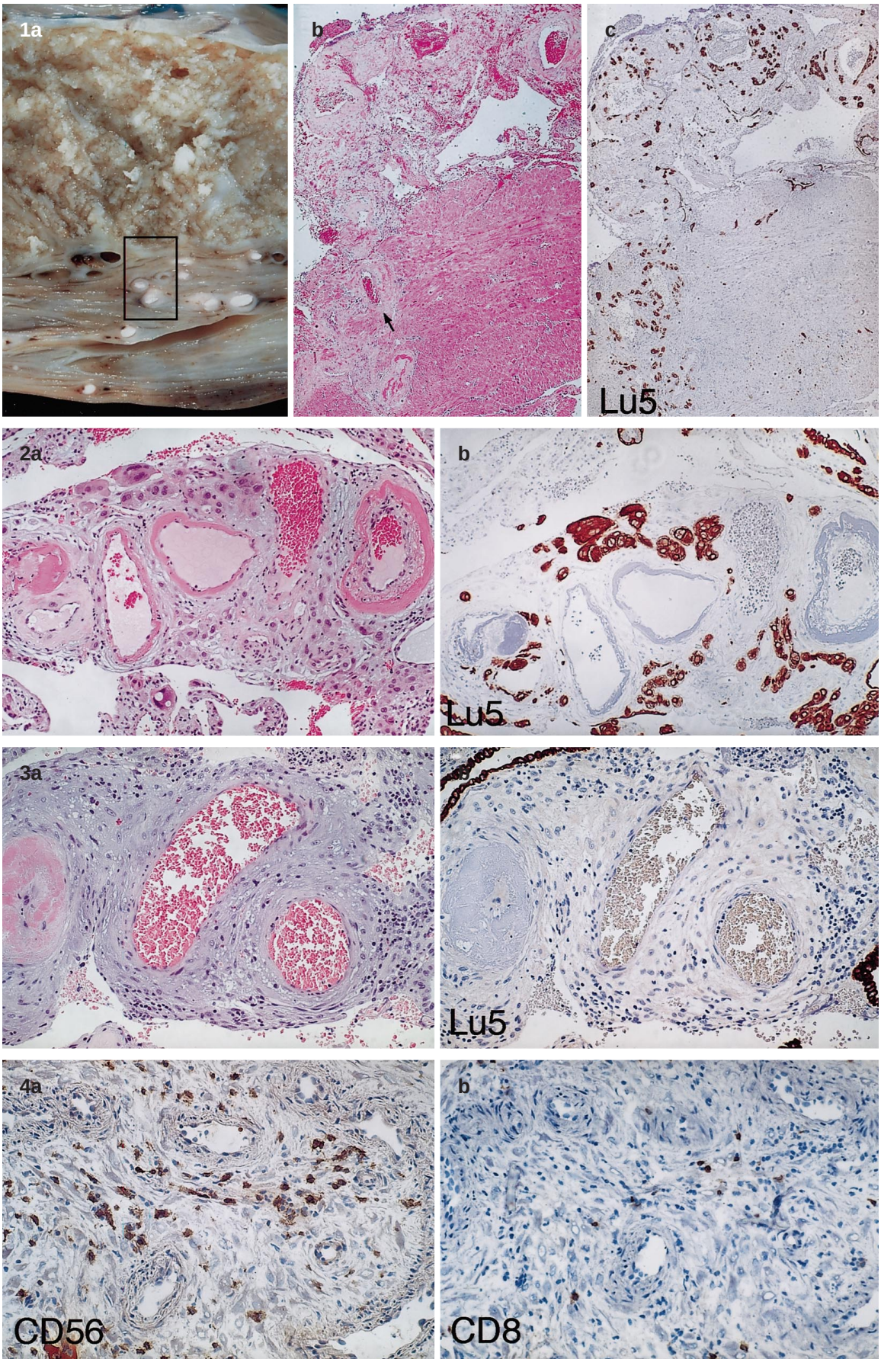
Examination of the multiple tissue fragments from the placental bed always started with the identification of trophoblast infiltration of the decidua, as this gives evidence of the placental bed proper. With the biopsy technique used an average of 6.2 spiral arteries were obtained from each patient. In control cases, about 40% of the spiral arteries remained in an unchanged state (intact muscle coat), while about 40% were transformed physiologically (disappearance of muscle). The ratio of transformed to unchanged arteries was thus about 1:1. Unchanged arteries had a lumen of 22 µm in diameter and were devoid of trophoblast. In physiologically trans-

Fig. 1a–c Uterus, placenta and feto-maternal interface ("placental bed"). **a** Maternal vascular system injected with a white dye. The *box* indicates decidua and inner layer of myometrium with a large tortuous spiral artery. **b** The myometrial segment of the artery (*arrow*) usually is not present in a specimen taken with a curette. ×20 **c** In normal pregnancy, the physiologically transformed spiral arteries are surrounded and infiltrated by fetal trophoblast visualized immunohistochemically with cytokeratin antibody Lu5. ×20 ▶

Fig. 2a, b Lack of trophoblast invasion into spiral arteries. **a** Complete loss of muscle tissue within vessel walls occurs in physiological transformation; however, the deposition of eosinophilic material (in the haematoxylin-eosin stain) and the occurrence of foam cells is a pathological finding termed acute atherosis. **b** The cytokeratin stain demonstrates trophoblast, which is often multinucleate and has failed to invade vessel walls. ×100

Fig. 3a, b Decidual inflammation associated with severely reduced total trophoblast. **a** Spiral artery in a curettage biopsy from the placental bed, with acute atherosis and heavy infiltration of the decidua by maternal lymphocytes. **b** The cytokeratin stain is only positive in cells of the uterine glands (positive internal control) and thus reveals the complete absence of trophoblast. ×100

Fig. 4a, b Lymphocytes within the decidua of a growth retarded fetus. **a** In this section spiral arteries have not been transformed; trophoblast is reduced and lymphocytes augmented. Typing of lymphocytes is achieved immunohistochemically on frozen sections with anti-CD56, showing an increased number of uterine NK cells. **b** Serial sections are used to count $CD8^+$ T cells. ×250


1a
b
c
Lu5
2a
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Lu5
3a
Lu5
4a
CD56
b
CD8

formed arteries, the diameter of the lumen was increased by 5 times to a median of 110 µm and trophoblast cells were abundant within the walls (Fig. 1c).

With pre-eclampsia, the diameters of unchanged and transformed spiral arteries did not differ from those in normal pregnancy. However, there was more variation in the diameter of arteries with segmental widening and characteristic narrowing by eosinophilic refractile depositions (Fig. 2). This acute atherosis affected less than 10% of all cross sections of spiral arteries found within the tissue of a placental bed biopsy from a normal pregnancy. With pre-eclampsia, acute atherosis affected up to 30% of all cross sections, in addition to which, the number of arterial cross sections lying closely together and thus belonging to one artery was increased (6.9 cross sections on average, compared with 3.5 in normal pregnancy). This indicates a more tortuous course of such arteries in pre-eclampsia. The ratio of transformed to unchanged spiral arteries was reduced, the difference reaching significance ($P<0.05$) once pre-eclampsia was associated with intrauterine growth retardation. Within the walls of transformed arteries, trophoblast cells were always significantly reduced ($P<0.05$). Specifically, the trophoblast was either completely absent or only segmentally infiltrating about one quarter to a half of the vessel circumference, while in a normal pregnancy trophoblast infiltration is found in the full circumference of spiral arteries.

More distant from the arteries two patterns of trophoblast behaviour could be seen, featuring either normal numbers with an increased percentage of multinucleated trophoblast cells (Fig. 2) or severe reduction and concomitant inflammation (Fig. 3).

In normal pregnancies the placental bed contained macrophages and lymphocytes, which were not evenly distributed, but accumulated focally. The proportion of decidual macrophages to total lymphocyte numbers was about 1:1 (not shown). Analysis of frozen sections revealed that decidual lymphocytes were about 40% $CD56^+$ uterine NK cells and 60% $CD3^+$ T lymphocytes; about 30% of these were $CD8^+$ T cells (Fig. 4).

With pre-eclampsia (maternal symptoms, normal fetal growth) there was no difference in the total numbers of uterine NK cells ($CD56^+$) and lymphocytes ($CD3^+$). However, $CD8^+$ T cells were increased to about 50% of all $CD3^+$ cells. Pre-eclampsia with fetal growth retardation was accompanied by an increase in the total number of uterine NK cells and $CD8^+$ T cells. The ratio of decidual $CD8^+$ T cells increased further, to 70% (Fig. 5).

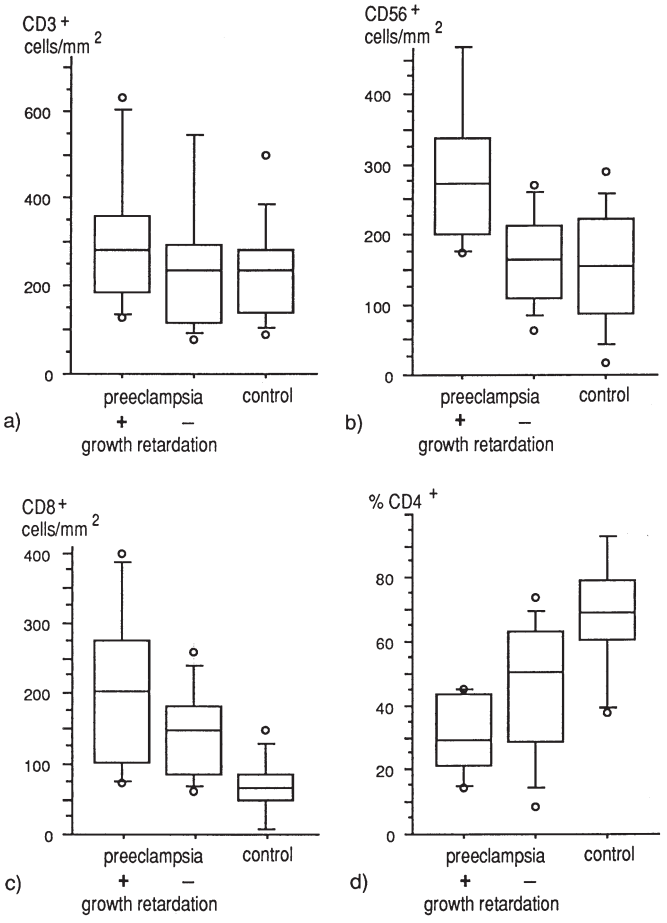

Fig. 5a–d Lymphocyte numbers in the decidua. (*Boxes* show median and contain 50% of all measurements). **a** $CD3^+$ T cells do not show a difference between healthy pregnancy and disease. **b** $CD56^+$ uterine NK cells are increased ($P<0.05$) with fetal growth retardation but not with pure pre-eclampsia. **c** $CD8^+$ T cells are significantly increased ($P<0.05$) in the preeclamptic state compared with healthy pregnancy. **d** The percentage of $CD4^+$ T cells drops, and the difference between pure pre-eclampsia and pre-eclampsia with associated fetal growth retardation is again significant ($P<0.05$)

Discussion

About 40 years ago, pathological changes in spiral arteries were described as diagnostic for and causally related to pre-eclampsia [2]. The studies were done on excision biopsies from the placental bed, and on average only about one artery per case was examined. Since about 100 arteries lead to a fully grown placenta, no information could be gleaned on the relative proportions of arteries that are physiologically transformed, remain unchanged, or exhibit degeneration in the form of acute atherosis. With an average of 6.2 spiral arteries in our biopsy material, we were surprised to note that in a normal pregnancy about 40% of the spiral arteries remain in an unchanged state. This might constitute a reserve pool, which can be used for compensation in cases of circulatory deficiencies. We furthermore found up to 10% of degenerated spiral arteries, either featuring foam cells or exhibiting acute atherosis even in normal pregnancies. Thus, the finding of a single artery with acute atherosis is not pathognomonic for pre-eclampsia. Our study also shows that tissue obtained with a curette from the placental site exhibits distinct differences between normal and disease states and is thus a suitable material for further studies.

The trophoblast content within spiral arteries leading to the placenta of growth retarded fetuses was found to be severely reduced. This was correlated with significant

differences in the architecture of these vessels; arteries had a smaller diameter and followed a more tortuous course than the physiologically transformed arteries. This was revealed by a greater number of cross sections per artery, as has also recently been reported by others [14]. There are two different patterns of trophoblast behaviour in the defective placentation that occurs in pre-eclampsia. One is impeded migration, which is characterized by visible trophoblast cells in normal or even increased numbers in the decidua, but not within the walls of spiral arteries. The second pattern is a severe reduction of trophoblast cells in combination with a strong increase of maternal lymphocytes and uterine NK cells. In this instance, some sort of 'inflammatory reaction' seems to reduce the total amount of trophoblast tissue. Acute atherosis, which occurs in poorly transformed vessels, may be a secondary phenomenon related to the lack of trophoblast invasion.

It is noteworthy that each and every placental bed contains a large number of lymphocytes and macrophages. The cellular composition of this physiological inflammation is perhaps essential for the temporal acceptance of the fetal allograft. Pre-eclampsia exhibits a significant decrease in the CD4/CD8, ratio which strengthens the concept of preeclampsia as an immunological disease [1, 6, 8, 13]. However, a significant increase of $CD56^+$ uterine NK cells is found only when fetal growth retardation is present. It is not clear whether this is only due to a more advanced disease state or whether there is a different pathway leading to pre-eclampsia from the onset. The results of our study indicate that pre-eclampsia in its early stages can be regarded as an altered balance between trophoblast invasion and maternal lymphocytic defence against the fetal allograft.

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