Stimulated Hofbauer Cells in the Placental Villi from Patients with Second-Trimester Abortions

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The purpose of this study was to characterize the ultrastructural and enzyme-histochemical features of Hofbauer cells in the placental villi of second-trimester abortions. Hofbauer cells in the abortive placenta had more numerous cytoplasmic processes and more prominent phagosomes as compared to gestational age-matched normal control

placenta. Acid phosphatase activity was demonstrated in both the lysosomes and on phagosomal membranes of these cells. Our results indicate that Hofbauer cells in second-trimester abortions are activated or stimulated *in situ*. These activated Hofbauer cells might play important roles in the pathophysiology of preterm parturition.

Key words: Hofbauer cells, Placenta, Abortion, Acid phosphatase

I. Introduction

Intrauterine infection is a major cause of preterm parturition (second-trimester abortion and preterm delivery) [2, 7]. Human placenta consists of two parts, chorioamnion (fetal membrane) and placental villi. Chorioamnionitis (CAM), in which abundant inflammatory cells, mainly maternal polymorphonuclear leukocytes (PMNs) or mononuclear phagocytes, infiltrate into the fetal membranes, is frequently observed during infection [2, 7]. The large number of PMNs and phagocytes present might not only kill bacteria but also attack the host itself (mother) by releasing superoxide, granulocyte elastase and cytokines [2, 7]. Activated PMNs may be a causal factor of preterm parturition [12, 14]. Villitis, on the other hand, is a relatively rare phenomenon associated with preterm parturition [2, 7, 9]. Though infiltration of the inflammatory cells or infectious organisms is seldom observed in the placental villi or in the intervillous space, toxins induced by inflammation or infection may accumulate here [2]. Thus it remains to be elucidated whether inflammatory cells are activated or stimulated in the placental villi in preterm parturition.

Hofbauer cells are believed to be fetal tissue macrophages and possess potent phagocytic activity [4–6, 20, 21]. During the passage of blood components between

the mother and fetus, the human placenta prevents undesirable substances, such as bacteria or toxins, from passing to the fetus. Since infiltration of maternal inflammatory cells into the placental villi is rare, Hofbauer cells might be involved in defense against infectious stimuli or undesirable substances. Are Hofbauer cells of the placental villi activated in patients with preterm parturition? Do these cells have any roles in the pathophysiology of preterm parturition? In order to settle these questions, we investigated the morphological characteristics of Hofbauer cells in the placenta of second-trimester abortions. We employed ultrastructural enzyme-histochemistry of acid phosphatase (ACP) as a marker enzyme for lysosome, as well as transmission electron microscopy.

II. Materials and Methods

Subjects were five Japanese women, who aborted at 19–21 weeks of gestation. They were clinically diagnosed with infection, such as elevated CRP level ($1.0 \pm 1.2 < \text{mean} \pm \text{SD} > \text{mg/dl}$), leukocytosis ($15.3 \pm 3.0 \times 10^3/\mu\text{l}$) and low grade fever ($37.0\text{--}37.6^\circ\text{C}$), and had regular uterine contractions of at least 10-min intervals. Excluded were cases with clinical histories of cervical incompetency or premature rupture of the membranes (PROM). Patients received tocolysis treatment by β_2 -agonist and antibiotic therapy, without success. Routine histological examination revealed the presence of CAM, but infiltration of inflammatory cells into the villi (villitis) was not observed. Five pregnant women, matched for gestational

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ages, whose clinically normal pregnancies were interrupted by legal abortion, constituted the normal controls. These women did not show clinical findings of infection (CRP; 0.1 ± 0.02 mg/dl, WBC; $9.0 \pm 1.7 \times 10^3/\mu\text{l}$, body temperature; $36.0\text{--}36.9^\circ\text{C}$).

After obtaining informed consent, 6 randomly selected pieces of macroscopically normal placental tissues were obtained from the maternal surface within 3 min of delivery. Tissues were fixed in 2.0% glutaraldehyde in a cacodylate buffer (0.1 M, pH 7.4) for 60 min. After being washed in the cacodylate buffer, the pieces were sectioned ($40\ \mu\text{m}$) on a freezing microtome. Sections were incubated for 30 min at 37°C in a lead nitrate medium (45 mM acetate buffer [pH 5.0], 3 mM lead nitrate, 10 mM beta-glycerophosphate) [11] or in a cerium medium (100 mM acetate buffer [pH 5.0], 1 mM beta-glycerophosphate, 2 mM cerium chloride) [15] for cytochemical detection of ACP activity. To confirm the specific cytochemical detection of ACP, control experiments were also performed as follows: (1) substrate (beta-glycerophosphate) was omitted from the medium; (2) sections were preheated (65°C , 30 min) and then incubated in the medium. Sections were also fixed in 1% buffered osmium tetroxide for 60 min, dehydrated, and embedded in epoxy resin for TEM studies. Ultrathin sections were prepared and observed under TEM. We analyzed 300 Hofbauer cells (50 per each specimen, 6 randomly taken specimens per case) in immature intermediate villi in each placenta. Data was analyzed statistically using unpaired Student's *t*-test.

III. Results

Light-microscopic observations

Hofbauer cells were observed in the villous stroma of the intermediate placental villi. Deposits indicating ACP activity were observable in these Hofbauer cells in normal (Fig. 1a) and abortive placenta (Fig. 1b). At light-microscopic level, Hofbauer cell number, ACP intensities and its localizations apparently did not differ between abortive and normal placenta.

Electron-microscopic observations

In placenta obtained from normal pregnancies, Hofbauer cells possessed cytoplasmic processes, membrane-bound electron-lucent vacuoles (phagosomes) and electron-dense granules (Fig. 2a). These findings were in agreement with the previous observations made by other researchers [3–5, 8]. ACP activity was observed in the cytoplasmic granules (Fig. 2b, c), indicating that these granules were lysosomes. Trophoblasts also possessed ACP activity in their lysosomes, as previously reported by our group (Fig. 2b) [13]. Omission of the substrate from the medium and preheating of the sections abolished enzyme activity (data not shown). Lead nitrate and cerium methods gave identical results (Fig. 2b, c).

In abortive placenta, cytoplasmic processes and phagosomes of Hofbauer cells were more prominent and were more numerous (Fig. 3a). In most cells, ACP activity was clearly visible in both the lysosome and the luminal

Fig. 1. Human placental villi. Light-microscopic localization of ACP activity in normal control placenta at 21 weeks of gestation (a) and abortive placenta at the same gestational age (b). Bar indicates $100\ \mu\text{m}$. a. Deposits indicating ACP activities were observable in the cytoplasm of Hofbauer cells in the villous stroma (arrows) from normal placenta. Activity was also seen in the syncytiotrophoblastic layer (S). ivs, intervillous space. b. ACP activity was positive in Hofbauer cells from this abortive placenta. Enzyme intensity, its distributions and Hofbauer cell number apparently did not differ from normal controls.

Fig. 2. Transmission electron micrographs indicating ultrastructure (**a**) and ACP localization (**b**, **c**) of Hofbauer cell in normal placenta of 21 weeks of gestation. Bar indicates 1 μ m. **a.** Hofbauer cell possesses cytoplasmic processes, electron-lucent vacuoles and electron-dense granules. Granules (*) are shown in the upper inset at a higher magnification. Lower left inset indicates the electron-lucent vacuole marked by the arrow. **b.** ACP activity of Hofbauer cell from normal placenta. Lead nitrate method is used. Electron-dense reaction products indicating ACP activity are visible in the cytoplasmic granule (arrowhead) of Hofbauer cells. Precipitates can be observed in lysosomes (arrows) of the syncytiotrophoblasts (S) as well. The number of lysosomes per Hofbauer cells is variable. The cell in this micrograph possesses only one lysosome in this field. **c.** ACP activity of Hofbauer cell from normal placenta, using cerium method. Precipitates indicative of ACP activity can be seen in the granules (lysosomes) of Hofbauer cell.

side of the phagosomal membrane (Fig. 3b, c). We analyzed 1,500 Hofbauer cells (300 cells per placenta $\times$ 5 placentas) from abortive placenta, and 1,500 from normal one. The occurrence of Hofbauer cells, whose

phagosomal membranes showed positive ACP staining in abortive and normal placenta were 852/1,500 (56.8%) and 225/1,500 (15.0%), respectively, statistically significant differences ($p < 0.01$). Occasionally the electron-dense

Fig. 3

granules (lysosomes) adhered to the phagosomes (Fig. 3c). In both the abortive and normal subjects, there were no significant differences in the morphological features and ACP staining pattern of Hofbauer cells within the same placenta. In other words, there was no variation in findings noted among specimens taken from the same placenta; thus these sections are believed to be representative findings for the various placental specimens.

IV. Discussion

Intrauterine infection is a key factor causing preterm delivery [2, 7]. Infection within the intrauterine environment possibly may lead to preterm parturition in second-trimester pregnancy in a similar manner to preterm delivery. Hofbauer cells are believed to be fetal macrophages [4–6, 20, 21], and they are numerous in the immature villi of second-trimester placenta [4]. We hypothesized that these cells might have pathophysiological significance in infection-related second-trimester abortions. The purpose of the present study is to characterize Hofbauer cells in the placental villi obtained from second-trimester abortions and to compare their morphology to those from normal pregnancies.

Previously, it has been reported that the most striking aspects of Hofbauer cells were electron-lucent vacuoles (phagosomes), cytoplasmic processes and intracytoplasmic electron-dense granules [4, 8]. These granules have been thought to be lysosomes [4, 8], but direct evidence has been lacking. Fox and Kharkongor [10] reported ACP positive cells in the villous stroma, but there have been no detailed ultrastructural studies reported to date. In the present study, we have demonstrated ACP activity to be localized in the intracytoplasmic granules of Hofbauer cells in the second-trimester placental villi for the first time. As ACP is believed to be confined and packed in the lysosomes and as it is widely used as a marker enzyme of lysosomes in other tissues [1], the intracytoplasmic granules observed in Hofbauer cells are also believed to be lysosomes. Hofbauer cells, therefore, possess the character of mononuclear phagocytic cells (tissue macrophages), as previously suggested by other researchers [20, 21].

Macrophages, when exposed to bacteria or inflammatory stimuli, form marked cytoplasmic processes and phagocytose these substances. Lysosomes fuse with phagosomes and release lysosomal enzyme (ACP) into a phagocytic vacuole [17]. In Hofbauer cells obtained from abortions, cytoplasmic processes and phagosomes were

more evident than from normal pregnancies. ACP histochemistry revealed that lysosomes were situated very near to phagosomes, and adhesion of lysosomes to phagosomes was occasionally observed. Furthermore, ACP activity was strongly positive on the luminal side of the phagosomal membrane. All these observations provide strong evidence that Hofbauer cells were either undergoing phagocytosis, activated, or stimulated. Intrauterine infection, including CAM, has been suggested to be a major causal factor of preterm parturition [2, 7]. In the present study, pathological examination revealed the presence of CAM in all patients, who all presented clinical signs of infection. Further, Hofbauer cells in abortive placental villi exhibited morphological and enzyme-histochemical evidence of being activated *in situ* without evidence of infiltration of maternal PMNs or maternal macrophages to the villi. Thus, it is reasonable to assume that Hofbauer cells, which are believed to be of fetal origin [4, 6], may react to infectious stimuli.

What is the pathophysiological significance of this Hofbauer cell activation? We propose two contrasting hypotheses. Hofbauer cells, when activated, may act as a barrier in the transmission of infection from mother to fetus. Ascending bacterial infections cause CAM, and then possibly intervillitis (infection of villi beneath the placental chorionic plate). Bacteria or other infectious substances entering the intervillous space [2], and Hofbauer cells, situated between this space and fetal blood circulation, may phagocytose and digest these undesirable substances. In this sense, Hofbauer cells may play a role in protection against infection. Activated Hofbauer cells, on the other hand, may act adversely on the host (mother). These cells have high thromboxane A₂ activity [19], and produce cytokines (interleukin-1 beta) [16]. Production of collagenase [18], elastase and reactive oxygen species has also been reported in tissue macrophages [17]. These substances might induce cervical maturation or start myometrial contraction, leading to premature onset of labor. We have already reported that maternal activated PMNs might adversely effect preterm labor [14].

In conclusion, ultrastructural and enzyme-histochemical examinations have demonstrated that Hofbauer cells in the placental villi are activated or stimulated in patients with second-trimester abortions. This activation of Hofbauer cells may be of considerable importance in the pathophysiology of second-trimester fetal wastage. There are several substances such as cytokines, growth factors, and other specific antigens of inflammatory cells, which

Fig. 3. Electron micrographs demonstrating ultrastructure (a) and ACP activity (b, c) of Hofbauer cell in abortive placenta of 21 weeks of gestation. Bar indicates 1 μ m. **a.** Phagosomes and cytoplasmic processes appear more prominent in Hofbauer cell from abortive placenta. **b.** ACP activity of Hofbauer cell from abortive placenta. Lead nitrate method is employed. Electron-dense reaction products indicative of ACP activity can be seen in the lysosomes. **c.** ACP activity of Hofbauer cell from abortive placenta, using cerium method. An area indicated by an arrow in the inset is shown in this electron micrograph at a higher magnification. Precipitates indicating ACP activity are clearly observed on the phagosomal membrane (arrows). Asterisk (*) indicates ACP positive lysosome, which appears to adhere or fuse to the neighboring phagosome.

are differently expressed at different stages of macrophage activation [17]. Immunohistochemical or biochemical study using these substances may shed further light on the functional significance of Hofbauer cell activation in preterm parturition.

V. References

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