## Bizarre hypertrophy of vascular endothelial cells in rhesus monkey endometrium: experimental induction and electron microscopical characteristics

## H.-W. DENKER<sup>1</sup>, A. C. ENDERS<sup>2</sup> and S. SCHLAFKE<sup>2</sup>

<sup>1</sup>Abteilung Anatomie der Rheinisch-Westfälischen Technischen Hochschule Aachen and <sup>2</sup>Department of Human Anatomy, University of California School of Medicine, Davis (CA, USA)

During the initial phase of embryo implantation in the uterus, the endometrial vascular bed is well known to show increased permeability to high molecular weight substances (proteins), at least in rodents where prostaglandins are thought to be involved in mediating this change (KENNEDY, 1979). Surprisingly little is known about ultrastructural details of the changes that must take place in the vascular wall, and information is particularly limited in case of primates including the human (ANCLA and DE BRUX, 1964; HARRIS, 1983; SHEPPARD et al., 1983).

In the rhesus monkey unusually large cells are found in the endothelial layer of the endometrial terminal vascular bed at implantation sites, giving these vessels a glandlike appearance. This phenomenon was described light microscopically by WISLOCKI and STREETER (1938) and HISAW and coworkers (see DALLENBACH-HELLWEG et al., 1966) but has received little attention and remained largely unknown. On the other hand there is confusion concerning the identity of the cells and more recent publications have favored the view that these are not modified endothelial cells but intravascularly migrating cytotrophoblast, particularly in the human and the hamster (HARRIS and RAMSEY, 1966; BUREK et al., 1979; DE WOLF et al., 1980; HATA et al., 1981; PIJNENBORG et al., 1981; RAMSEY and HARRIS, 1966).

In order to unequivocally eliminate the trophoblast from the system and to make sure that the enlarged cells are indeed modified endothelial cells, we have tried to elicit the modification of the endothelium by a non-specific traumatic stimulus in nonpregnant, cycling females.

In brief, 5 sexually mature, regularly cycling female rhesus monkeys were included in the study. The traumatic stimulus was provided by a 00 silk thread which penetrated the whole uterine wall and was placed at laparotomy done at day 17-21 of the cycle (6–10 days after the preovulatory estrogen peak determined by RIA; PARKIN and HENDRICKX, 1975). Three of the animals received progesterone supplementation (10 mg once daily) during the luteal phase in order to prolong the cycle. 5–9 days after stimulation, uterine biopsies were taken. Tissue was fixed by immersion in 2% glutaraldehyde-2% formaldehyde in 0.1 M phosphate buffer and processed for routine embedding in Araldite for transmission electron microscopy.

The most obvious change induced by thread traumatisation in the endometrium was the so-called "epithelial plaque reaction", a grotesque metaplasia of the uterine epithelium which is normally induced at embryo implantation sites in the rhesus monkey (WISLOCKI and STREETER, 1939; ROSSMAN, 1940; MARSTON et al., 1971) and which will be described in a subsequent communication. In our experiments, a plaque reaction as well as vascular endothelial modifications were only induced in the immediate vicinity of the thread and only in those animals which had received progesterone. These vessels resembled those found at implantation sites: wide, sinusoidal vessels with wall characteristics of capillaries or postcapillary venules, showing considerably thickend endothelium as never seen in any phase of the regular cycle (Figs. 1 + 2).



Fig. 1. Unmodified endothelium, monkey no. 17387, thread traumatization but no progesterone supplementation, day 11 after preovulatory estrogen peak.  $\times 24,000$ .

Fig. 2. Hypertrophying endothelium, monkey no. 6134, thread traumatization and progesterone supplementation, day 15 after preovulatory estrogen peak.  $\times 24,000$ .

Two types of those endothelial cells could be distinguished: 1. Lightly staining cells with only few organelles, sparse ER, often rich in glycogen; 2. cells with dense ground cytoplasm, very rich in widend cisterns of rough ER, sometimes somewhat reminding of plasma cells. In both cell types, Weibel-Palade bodies were rare, but present; transport vesicles rare; no fenestrations nor interendothelial gaps; junctions commonly characterized by an intermembrane space of aproximately 40 Å (possibly zonular) as known from other endothelia, while (probably macular) occluding type junctions were more rare. The basal lamina was mostly multilayered, mitoses were frequent.

The experiments described here allow us to exclude unequivocally a trophoblastic nature of the intimal cells. Comparable endothelial modifications are apparently not found after endometrial traumatisation in the baboon (WHEELER et al., 1983) but lesser degrees of endothelial hypertrophy can be seen at early human implantation sites (material of the Carnegie Collection, own observations), and have been reported to occur in various other species (DALLENBACH-HELLWEG et al., 1966).

It has previously been postulated that the endothelial proliferation in monkey endometrium is dependent on relaxin liberated from endometrial granular cells (DALLEN-BACH-HELLWEG et al., 1966). We cannot exclude that the progesterone effect which we observed may have been an indirect one. Estrogen receptors have been described in endothelial cells (BUONASSISI and COLBURN, 1980) but little is known about progesterone receptors in endometrial endothelia. The observed modifications do not seem to be typical for any of the known pathological phenomena, at least as described in other species (CASLEY-SMITH, 1980; MASON and BALIS, 1980) nor for the action of mediators like histamin (MAJNO et al., 1969).

High endothelia are found normally in specialized postcapillary venules in the spleen and lymph knodes (WENK et al., 1974; ANDERSON et al., 1976) and pathologically in the terminal vascular bed in granulomas (SMITH et al., 1970) and are thought to regulate lymphocyte penetration.

The structural modification of endothelial cells which appears to be much more obvious in the rhesus monkey than in other species may help to study functional changes in the terminal vascular bed in the endometrium which may involve altered permeability for either white blood cells or for plasma proteins, as typical for embryo mplantation sites.

The authors wish to thank Mr. H. BOFFIN for skilful EM work, the staff of the california Primate Research Center, Davis (CA), for help with the monkey experiments, and Ms. G. BOCK and Ms. G. MATHIEU for darkroom work and secretarial help. This investigation was supported by Stiftung Volkswagenwerk Adademiestipendium 36 552, Deutsche Forschungsgemeinschaft grant De 181/9-5 and NIH grant RR 00169 to Dr. A. HENDRICKX.

ANCLA, M., DE BRUX, J.: Étude au microscope électronique des artérioles spiralées de l'endomètre humain (Note préliminaire). Ann. Anat. pathol. (Paris) 9, 209–222 (1964)

ANDERSON, N. D., ANDERSON, A. O., WYLLIE, R. G.: Specialized structure and metabolic activities of high endothelial venules in rat lymphatic tissues. Immunology **31**, 455–473 (1976)

BUONASSISI, V., COLBURN, P.: Hormone and surface receptors in vascular endothelium. Adv. Microcirc. 9, 76-94 (1980)

BUREK, J. D., GOLDBERG, B., HUTCHINS, G., STRANDBERG, J. D.: The pregnant syrian hamster as a model to study intravascular trophoblasts and associated maternal blood vessel changes. Vet. Pathol. 16, 553-566 (1979)

- CASLEY-SMITH, J. R.: The response of the microcinculation to inflammation. In: The Cell Biology of Inflammation (G. WEISSMANN, ed.), Handbook of Inflammation, Vol. 2 (L. E. GLYNN et al., series-eds.). Elsevier/North-Holland Biomedical Press, Amsterdam, 1980, pp. 53-82
- DALLENBACH-HELLWEG, G., DAWSON, A. B., HISAW, F. L.: The effect of relaxin on the endometrium of monkeys. Histological and histochemical studies. Amer. J. Anat. 119, 61-77 (1966)
- DE WOLF, F., DE WOLF-PEETERS, C., BROSENS, I., ROBERTSON, W. B.: The human placental bed: electron microscopic study of trophoblastic invasion of spiral arteries. Amer. J. Obstet. Gynec. 137, 58-70 (1980)
- HAMMERSEN, F.: Bau und Funktion der Blutkapillaren. In: Handb. allg. Pathol., Vol. III/7: Allgemeine Pathologie der Mikrozirkulation. Springer, Berlin, 1977, pp. 135–229
- HARBIS, J. W. S.: Intravascular trophoblast (Abstr.). J. Anat. 137, 792 (1983)
- HARRIS, J. W. S., RAMSEY, E. M.: The morphology of human uteroplacental vasculature. Carnegie Inst. Wash. Contributions Embryol. 38, 43-58 (1966)
- HATA, T., OHKAWA, K., UCHIDA, K., KAMITANI, N.: An ultrastructural study of penetration and replacement process by trophoblast cell into human maternal vessel (Jap.). Acta obstet. gynaec. jap. 33, 1917–1924 (1981)
- KENNEDY, T. G.: Prostaglandins and increased endometrial vascular permeability resulting from the application of an artificial stimulus to the uterus of the rat sensitized for the decidual reaction. Biol. Reprod. 20, 560-566 (1979)
- MAJNO, G., SHEA, S. M., LEVENTHAL, M.: Endothelial contraction induced by histamine-type mediators. An electron microscopic study. J. Cell Biol. 42, 647-672 (1969)
- MARSTON, J. H., KELLY, W. A., ECKSTEIN, P.: Deciduomal reaction induced in rhesus monkeys by tubal injection of arachis oil and by the presence of an intrauterine device. J. Reprod. Fert. 25, 451-454 (1971)
- MASON, R. G., BALIS, J. U.: Pathology of the endothelium. In: Pathobiology of Cell Membranes, Vol. II (B. F. TRUMP and A. U. AFSTILA, eds.). Academic Press, New York, 1980, pp. 425-471
- ORTMANN, R.: Histochemische Untersuchungen an menschlicher Placenta mit besonderer Berücksichtigung der Kernkugeln (Kerneinschlüsse) und der Plasmalipoideinschlüsse. Z. Anat. Entwickk-Gesch. 119, 28-54 (1955)
- PARKIN, R. F., HENDRICKX, A. G.: The temporal relationship between the preovulatory estrogen peak and the optimal mating period in rhesus and bonnet monkeys. Biol. Reprod. 13, 610-616 (1975)
- PIJNENBORG, R., ROBERTSON, W. B., BROSENS, I., DIXON, G.: Trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. Placenta 2, 71-92 (1981)
- RAMSEY, E. M., HARRIS, J. W. S.: Comparison of uteroplacental vasculature and circulation in the rhesus monkey and man. Carnegie Inst. Wash. Contrib. Embryol. 38, 59-70 (1966)
- Rossman, I.: The deciduomal reaction in the rhesus monkey (Macaca mulatta). I. The epithelial proliferation. Amer. J. Anat. 66, 277-365 (1940)
- SHEPPARD, B. L., HOURIHAN, H. M., DRUDY, L., BONNAR, J.: Electron microscopy of uterine vascular and perivascular changes through the menstrual cycle (Abstr.). J. Anat. 137, 795 (1983)
- SMITH, J. B., MCINTOSH, G. H., MORRIS, B.: The migration of cells through chronically inflamed tissues. J. Path. 100, 21-29 (1970)
- WENK, E. J., ORLIC, D., REITH, E. J., RHODIN, J. A.: The ultrastructure of mouse lymph knode venules and the passage of lymphocytes across their walls. J. Ultrastruct. Res. 47, 214-241 (1974)
- WHEELER, A. G., HURST, P. R., POYSER, N. L., ECKSTEIN, P.: Uterine histology and prostaglandin concentrations and utero-ovarian venous steroid and prostaglandin concentrations during the luteal phase of the menstrual cycle in baboons (Papio spp.) with or without an IUD. J. Reprod. Fert. 67, 35-46 (1983)
- WISLOCKI, G. B., STREETER, G. L.: On the placentation of the macaque (Macaca mulatta), from the time of implantation until the formation of the definitive placenta. Carnegie Inst. Wash. Contrib. Embryol. 27, 1-66 (1938)

Address: Prof. Dr. med. Dr. rer. nat. H.-W. DENKER, Abteilung Anatomie der Medizinischen Fakultät an der Rheinisch-Westfälischen Technischen Hochschule Aachen, Melatener Straße 211, D - 5100 Aachen.