Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *86*, Suppl. III, 23-25, 1991

EXTRACELLULAR MATRIX OF THE MOUSE ENDOMETRIUM DURING DECIDUALIZATION

P. A. ABRAHAMSOHN; TELMA M. T. ZORN & SERGIO F. OLIVEIRA

Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, 05508 São Paulo, SP, Brasil

Decidualization consists of a transformation of the loose connective tissue of the endometrium into an epithelioid structure called decidua. This event occurs during embryo-implantation in several groups of mammals.

In the mouse, embryo-implantation begins on the fifth day of pregnancy. On this occasion the first decidual cells can be observed in the antimesometrial stroma of implantation sites.

The decidual cells originate from the transformation of spindle-shaped fibroblastic cells of the endometrial stroma. This transformation involves cell proliferation, polyploidization, remarkable modifications of cell shape and contents as well as formation of intercellular junctions (Abrahamsohn, 1983, 1989; Finn & Lawn, 1967; Weitlauf, 1988). As a result of the progressive decidualization of the stroma, several degrees of cell transformation can be observed in the endometrium: the cells which are nearest to the embryo are more differentiated (mature decidual cells) whereas the cells present near the myometrium maintain fibroblastic features. Cells with intermediate characteristics (predecidual cells) are present between the population of mature decidual cells and the fibroblasts (Lawn et al., 1971).

Decidualization is associated with remarkable modifications of the extracellular space and of components of the extracellular matrix. The width of the extracellular space decreases as decidualization advances. Thus, narrow spaces exist betweem mature decidual cells and wide spaces between the fibroblasts of the periphery of the endometrium.

The arrangement of fibronectin changes during decidualization in the rat, as shown by immunocytochemistry (Grinnel et al.,.1982). Whereas prior to decidualization fibronectin completely surrounds the endometrial fibroblasts, during decidual transformation it appears as patches around the cells (Grinnell et al., 1982). In humans, on the other hand, fibronectin is seen to surround each decidual cell (Kisalus et al., 1987). Decidualization in humans is accompanied by the deposition of a basement membrane-like material around each decidual cell (Lawn et al., 1971). It has been demonstrated by immunocytochemistry that laminin, heparan sulfate and collagen types IV and V are present in this structure (Kisalus et al., 1987; Wewer et al., 1986). It is known that these molecules are components of true basement membranes.

We have been studying in our laboratory modifications of endometrial extracellular matrix components during decidualization in mice. The main results which were obtained are summarized below.

GROUND EXTRACELLULAR MATRIX

Ultrastructural cytochemical studies were done on mouse endometria fixed with glutaraldehyde added with cationic dyes such as safranine O, alcian blue, and ruthenium red. These substances are known to preserve proteoglycans in tissues, allowing the analysis of the arrangement of proteoglycans and their relationship with cells and other components of the extracellular matrix. These studies showed that the network of proteoglycans was much more compact in the intercellular space of nondecidualized endometria than of decidualized ones. This network of proteoglycans is connected both with cell surfaces and collagen fibrils. On the fourth day of pregnancy, patches of a safranine O- and ruthenium red-positive material were observed on the surface of stromal cells that showed initial signs of decidualization. Interestingly, the same kind of patches is also found on the surface of predecidual cells from the fifth day of pregnancy on. A network of proteoglycans was frequently seen anchored to these patches. These patches are probably sites of deposition of heparan sulfate. Similar images were observed by Chen & Wight (1984) in cultured blood vessel wall. In this tissue, the stainability of the patches by cathionic dyes was removed after heparinase treatment. The images observed in decidual cells are very similar to those described as fibronexus by

Singer (1979). It is possible that these patches could be involved in events of cellular adhesion which begin on the fourth day of pregnancy in the mouse.

In the region of mature decidual cells the extracellular spaces are narrow and occupied mostly by thick collagen fibrils (Zorn et al., 1986). In this region proteoglycans were observed as an electron dense material which surrounded the collagen fibrils and, in many places, filled completely the narrow extracellular spaces. These data suggest a modulation of the contents of proteoglycans during decidualization. This hypothesis was confirmed by biochemical analysis of the types of glycosaminoglycans which are present in the mouse endometrium before and during decidualization. We found an increased amount of hyaluronic acid on day five of pregnancy, as compared to the amount present on days six and seven. Although no hyaluronic acid could be detected in virgin endometria, they showed the highest levels of sulfated glycosaminoglycans. On the other hand, the pregnant endometria had lower amounts of sulfated glycosaminoglycans; the relative proportion of these compounds did not change during decidualization. These data were complemented with a radioautographic study that showed that the fibroblasts of virgin endometria incorporate more radiosulfate than the decidual cells of pregnant endometria (Carvalho, 1989). These data aggree with the biochemical results described above. This study also revealed that the different regions of the pregnant endometrium exhibit different levels of synthesis of sulfated glycosaminoglycans (Zorn et al., 1990b).

FIBRILLAR EXTRACELLULAR MATRIX

Regarding connective tissue fibrils, one of the foremost events observed during decidualization of the mouse endometrium is a remarkable increase of the diameter of collagen fibrils (Zorn et al., 1986; Alberto-Rincon et al., 1989).

The average of the diameter of collagen fibrils in the endometrial stroma of virgin mice is about 50 nm. Such fibrils have regular profiles and are also present in the stroma of interimplantation sites (where decidualization does not occur) as well as in the periphery of the endometrium of implantation sites, which does not undergo decidualization.

On the other hand, the diameters of collagen fibrils that surround predecidual and principally decidual cells is increased, reaching more than 400 nm on the seventh day of pregnancy. Besides the diameter increase, the fibrils with enlarged diameter have very irregular profiles (Alberto-Rincon et al., 1989).

The diameter increase of the collagen fibrils in the pregnant mouse endometrium is clearly associated with the transformation of fibroblasts into decidual cells, as fibrils with largest diameters always surround the cells at more advanced stages of decidualization.

Several evidences point out to a participation of the decidual cells on the modifications of decidual extracellular matrix. Schlafke et al. (1985) have shown that the degeneration of the basement membrane that underlies the epithelium of the implantation crypt is caused by decidual cells.

We observed that endometrial fibroblasts phagocyte collagen fibrils during the periimplantation period (Zorn et al., 1986) and that thereafter decidual cells phagocyte collagen fibrils (Zorn et al., 1989). The demonstration of acid phosphatase activity in collagen-containing vacuoles of mouse decidual cells indicates that the internalization of the fibrils is followed by fusion of the vacuoles with lysosomes (Zorn et al., 1989). This results indicate that phagocytosis of collagen fibrils may be a feature common to all kinds of fibroblasts (Ten Cate & Freeman, 1974), a concept that may be applied to a fibroblast-derived cell such as the decidual cell. The data also suggest that phagocytosis of collagen is one of the mecanisms of remodeling of the mouse decidua. The remodeling of the endometrial stroma is an obligatory event which allows the expansion of the growing embryo.

An ultrastructural study on endometria fixed with glutaraldehyde added with tannic acid showed that collagen-containg phagosomes were very often associated with a well developed net of intermediate filaments. These vacuoles were frequently localized near the nucleus (Zorn et al., 1990a). Glasser & Julian (1986) described that besides producing vimentin the decidual cells synthesize a large amount of desmin and proposed this intermediate protein filament to be considered as a marker of decidualization. We do not know, however, which kind of intermediate filament is associated with phagosomes in decidual cells.

Electron microscopic radioautographic studies demonstrated that decidual cells are able to incor-

porate 3H-proline. At 1 hour after the injection of the radioactive aminoacid, most of the silver grains were concentrated over decidual cells. After 2, 6, and 24 hours, the silver grains were localized on the extracellular space, mostly over thick collagen fibrils (Oliveira et al., 1990). These results strongly suggest that, as in humans (Kisalus et al., 1987), the decidual cells of the mouse produce collagen. They also suggest that thick collagen fibrils are, at least in part, formed by aggregation of collagen molecules synthesized "de novo" by decidual cells.

Adding these results together it is reasonable to conclude that the decidua may prove to be a good model for the study of reciprocal interactions between cells and extracellular matrix components, in a tissue that undergoes fast differentiation and maturation.

REFERENCES

ABRAHAMSOHN, P. A., 1983. Ultrastructural study of the mouse antimesometrial decidua. *Anat. Embryol., 166*: 263-274.

ABRAHAMSOHN, P. A., 1989. Morphology of the decidua. p. 127-133. In K. Yoshinaga, *Blastocyst Implantation*, Adams, Boston.

ALBERTO-RINCON, M. C.; ZORN, T. M. T. & ABRAHAMSOHN, P. A., 1989. Diameter increase of collagen fibrils of the mouse endometrium during decidualization. *Am. J. Anat., 186*: 412-429.

CARVALHO, J. J., 1989. ***Incorporação de sulfato de sodio radioativo por células do endométrio de camundongas durante o período inicial de gestação***. PhD Thesis, Instituto de Ciências Biomédicas da USP, São Paulo, 85 p.

CHEN, K. & WIGHT, T. N., 1984. Proteoglycans in arterial smooth muscle cell cultures: an ultrastructural histochemical analysis. *J. Histochem. Cytochem., 32*: 344-357.

FINN, C. A. & LAWN, A. M., 1967. Specialized junctions between decidual cells in the uterus of the pregnant mouse. *J. Ultrastruct. Res., 20*: 321-327.

GLASSER, S. R. & JULIAN, J., 1986. Intermediate filament protein as a marker of uterine stromal cell decidualization. *Biol. Reprod., 35*: 463-476.

GRINELL, F.; HEAD, J. R. & HOFFPAUER, J., 1982. Fibronectin and cell shape *in vivo*: Studies on the endometrium during pregnancy. *J. Cell Biol., 94*: 594-606.

KISALUS, L. L.; HERR, J. C. & LITTLE, C. A., 1987. Immunolocalization of extracellular matrix and collagen synthesis in the first-trimester human decidua. *Anat. Rec., 218*: 402-415.

LAWN, A. M.; WILSON, E. W. & FINN, C. A. 1971. The ultrastructure of human decidual and predecidual cells. *J. Reprod. Fertil., 26*: 85-90.

OLIVEIRA, S. F.; NAGATA, T.; ABRAHAMSOHN, P. A. & ZORN, T. M. T., 1990. Incorporation of 3H-proline by mouse decidual cells: an ultrastructural radioautographical study (Abstract). 1º Simpósio Brasileiro sobre Matriz Extracelular. Itatiaia, p. 8.

SCHLAFKE, S.; WELSH, A. O. & ENDERS, A. C., 1985. Penetration of the basal lamina of the uterine luminal epithelium during implatation in the rat. *Anat. Rec., 212:* 47-56.

SINGER, I., 1979. The fibronexus: a transmembrane association of fibronectin-containing fibers and bundles of 5 nm microfilaments in hamster and human fibroblasts. *Cell, 16*: 675-685.

TEN CATE, A. R. & FREEMAN, E., 1974. Collagen remodeling by fibroblasts in wound repair. Preliminary observations. *Anat. Rec., 179*: 543-546.

WEITLAUF, H. M., 1988. Biology of implantation. In E. Knobil et al., *Physiology of Reproduction*, Raven Press, New York.

WEWER, U. M.; FABER, M.; LIOTTA, M. A. & ALBRECHTSEN, R., 1985. Immunochemical and ultrastructural assesment of the nature of the pericellular basement membrane of human decidual cells. *Lab. Invest., 53*: 624-633.

ZORN, T. M. T.; BEVILACQUA, E. M. A. F. & ABRAHAMSOHN, P. A., 1986. Collagen remodeling during decidualization in the mouse. *Cell Tissue Res., 244*: 443-448.

ZORN, T. M. T.; BIJOVSKY, A. T.; BEVILACQUA, E. M. A. F. & ABRAHAMSOHN, P. A., 1989. Phagocytosis of colagen by mouse decidual cells. *Anat. Rec., 225*: 96-100.

ZORN, T. M. T.; DE OLIVEIRA, S. F. & ABRAHAMSOHN, P. A., 1990a. Organization of intermediate filaments and their association with collagen-containing phagosomes in mouse decidual cells. *J. Struc. Biol., 103*: 23-33.

ZORN, T. M. T.; PINHAL, M. A. S.; NADER, H.; CARVALHO, J. J.; DIETRICH, C. P. & ABRAHAMSOHN, P. A., 1990b. Glycosaminoglycans during decidualization in the mouse (Abstract). 1º Simpósio Brasileiro sobre Matriz Extracelular. Itatiaia, p. 3.