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MINI REVIEW

GROWTH FACTORS IN PREGNANCY

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Abstract: The role of growth factors in pregnancy is a rapidly expanding subject. With the advent of molecular biological techniques more and more detailed information is available to the researcher. This review does not attempt to be exhaustive in its coverage of growth factors in pregnancy, rather it tries to give a brief taste of the possible roles that they may play in pregnancy by considering three specific factors, leukaemia inhibitory factor, colony stimulating factor-1 and vascular endothelial growth factor.

Key words : growth factors, implantation, LIF, CSF-1, VEGF, trophoblast

INTRODUCTION

Growth factors comprise various groups of polypeptides that mediate signalling between cells. They interact with specific receptors in the cell membrane of target cells, initiating intra-cellular signalling pathways. This can result in cell division, cell differentiation, protein synthesis or chemotaxis. Growth factors synthesised by the uterus or the placenta include the haematopoietic cytokines first identified on the basis of their ability to stimulate proliferation or differentiation of haematopoietic precursor cells within bone marrow (see Table I).

TABLE I: Growth factors produced in the uterus or placenta [For review see Pollard 1991, (2)](1).

Interleukins, TNFα, TGFβ family, CSFs (CSF-1, GM-CSF, G-CSF, stem cell factors).
LIF, VEGF, PIGF, EGF, c-kit.

There is now evidence for the involvement of many growth factors in the growth and differentiation of trophoblasts (for review see Pollard and Tabibzadeh) (1, 2). This article will discuss the possible roles of three of the best characterised growth factors; leukaemia inhibitory Factor (LIF), Colony Stimulating Factor-1 (CSF-1) and Vascular Endothelial Growth Factor (VEGF). The aim will be to illustrate some of the general principles regarding their modes of action that are emerging.

Implantation

Implantation begins with the invasion of the uterine epithelium and migration into the underlying stroma by trophoblast cells (see Fig. 1). Some of these cells form the placental villi (villous cytotrophoblast and syncytiotrophoblast) while extravillous trophoblast invade as far the inner third of the myometrium in a manner similar to tumour cell invasion. These cells differentiate either into sessile placental bed giant cells or destroy the media of maternal spiral arteries, converting them into distended sinusoidal sacs. This vascular conversion is essential for the maintenance of an adequate blood supply to the developing foetus. Disruption of this process may have severe consequences.

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Pre-eclampsia and IUGR are associated with inadequate invasion or premature differentiation of the invading trophoblast (3), whereas unrestrained invasion through the uterine wall, as seen in placental percreta can be lethal to the mother. Nutritionally deprived foetuses appear to suffer from a higher incidence of various diseases in later life, such as heart attacks and diabetes (4).

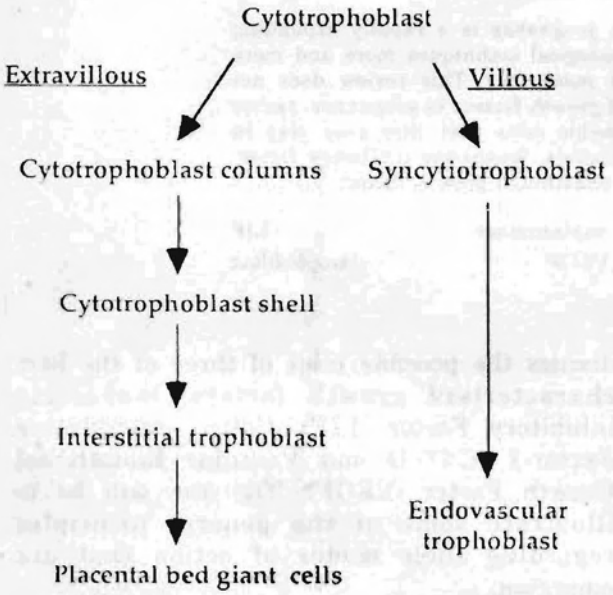

Fig. 1 : *Trophoblast differentiation* : Trophoblast may be considered a stem cell population capable of differentiating into a variety of cell types along two main pathways.

Clearly, defining the mechanism controlling trophoblast migration and differentiation would be an important step in understanding the aetiology of these conditions. The regulation of trophoblast differentiation involves a complex interaction between trophoblast and components of the maternal decidua. These include stromal cells, glandular epithelium and lymphoidal cells such as macrophages, T-cells and large granula lymphocytes (LGLs). This interaction is mediated at least in part by growth factors.

Leukaemia inhibitory factor (LIF)

LIF is a recently described cytokine produced by many cell types (5). It has been shown to have pleiotrophic effects, either suppressing or inducing differentiation depending upon the target cell type, and more recently, to play a vital role in implantation. The first evidence for the latter came when LIF mRNA expression was detected in uterine glands in pregnant mice only at the time of implantation (6). Experiments with transgenic 'knockout' mice have shown that female mice lacking a functional LIF gene, and hence unable to express LIF in the uterine glands on days 4-5, fail to undergo implantation. However, the blastocysts of these mice are viable and can implant and develop to term when transferred to wild type pseudo-pregnant recipients. This defect is corrected by local infusion of LIF into the uterus (7). This, coupled with the fact that LIF receptors occur on the inner cell mass (ICM) of preimplantation blastocysts (and LIF has profound effects on the differentiation of ICM cells), has led to the suggestion that the LIF generated by the glands acts directly on the pre-implantation embryo, and is vital to successful implantation.

Recently we have shown a similar pattern of LIF expression in women; LIF is secreted by the glandular epithelium in the mid-late luteal phase and the mRNA encoding the LIF receptor has been demonstrated in pre-implantation human blastocysts (8). LIF potentially has an important role in human implantation as it clearly does in mice. It will be particularly interesting to investigate LIF levels in infertile women in luteal endometrium. For a summary of the roles of LIF in implantation see Table II.

Colony stimulating factor-1 (CSF-1)

The mononuclear phagocytic growth factor CSF-1 was one of the first haematopoietic growth factors to be detected in the placenta, and its role is perhaps the best understood. The CSF receptor is the protooncogene product c-fms. CSF-1 is secreted by decidul glands throughout the first trimester, and CSF-1 receptor

TABLE II : The role of LIF in murine and human implantation.

Mouse	*Human*
LIF peptide secreted by glands on days 4-5 (just prior to implantation).	LIF peptide secreted by glands mid-late luteal phase.
Receptor expressed by blastocyst.	mRNA for receptor expressed by blastocyst.
LIF prevents differentiation of ICM cells.	?

(CSF-1R) is localised on trophoblasts, indicating a primary role in trophoblast regulation (see Table III). Immunocytochemical studies have shown CSR-1R expression is acquired in cytotrophoblast columns as it begins to proliferate and migrate and that CSF-1R is lost as the trophoblast differentiates to sessile placental giant cells (1). Support for the idea that CSF-1 may regulate the migration and differentiation of these cells has come from *in vitro* studies on trophoblasts, where it induces differentiation into syncytiotrophoblast-like cells (9).

TABLE III : CSF effects on human trophoblast *in vitro*.

- CSF causes trophoblast to differentiate into syncytiotrophoblast-like cells.
- Effect is abolished by antibodies to c-fms and CSF-1.
- CSF-1 induces hCG and hPL secretion.
- CSF-1 appears to be inducing differentiation along the villous developmental pathway.

In vivo studies in the mouse

Experiments in the mouse have produced information on the role of CSF-1 *in vivo*. The expression pattern is similar to humans, with CSF-1 as expected in the glands and CSF-1R on trophoblast. Trophoblast *in vitro* responds mitogenically to CSF-1, supporting the view that CSF-1 is a growth factor for trophoblast (10). Insight into the role of CSF-1 in implantation *in vivo* from the osteopetrotic *op/op* mouse (see Table IV), in which a natural mutation in the CSF-1 gene results in complete absence of CSF-1 production (11). Homozygous *op/op* matings are infertile, and the uteri of *op/op* females are found to contain very few macrophages compared to normal counterparts, and those that are present appear morphologically abnormal (1).

TABLE IV : Osteopetrotic Mice (op/op).

- No gene for CSF-1: lack monocytes and display skeletal abnormalities.
- Homozygous matings are infertile.
- Systemic CSF-1 does not correct fertility - local synthesis in uterus required.
- Uterus of op/op females is deficient in macrophages; suggests a key role for macrophages in implantation.

Taken together these results indicate a dual role for CSF-1 in the murine placenta. Firstly, a direct effect on proliferation or differentiation of mouse trophoblast, possibly due to CSF-1 produced within the uterine epithelium. Secondly, it implies a role in the recruitment and function of macrophages in the uterus during pregnancy. CSF-1 us a well known regulator of macrophage function in other tissue. Both of these roles require local production of CSF-1 as systemic administration of CSF-1 failed to correct the defect.

Since the pattern of expression of CSF-1 and CSF-1R in the human are largely consistent with those in the mouse, it is likely that CSF-1 plays a similar dual role in human trophoblast and uterine macrophage function (for summary see Table V).

Vascular endothelial growth factor (VEGF)

VEGF is a polypeptide first identified in 1989 (12). It interacts with specific cell surface receptors called flt and KDR (13, 14) activation

TABLE V : Actions of CSF-1.

•CSF-1 may act directly to regulate human and murine trophoblast migration and/or differentiation.
•CSF-1 is important in recruitment and regulation of macrophages on both sides of the placenta. CSF-1R is abundant on human foetal and maternal macrophages. In op/op mice lacking CSF-1, the decidual macrophages are deficient.
•Macrophage regulation requires local synthesis-with possible action by cell-cell contact.

of which mediates its action (see Table VI). It is mitogenic to vascular endothelial cells, and until recently endothelial cells were thought to be its sole target (15). It is widely distributed and is believed to be important for the maintenance of vascular integrity (15). VEGF is involved in new vessel growth (angiogenesis) both physiologically and as a result of injury, inflammation or hypoxia, and is believed to play a significant role in the pathogenesis of some cancers (16, 19).

TABLE VI : Actions of VEGF [summarised in Senger 1993] (19).

•Endothelial cell mitogen.
•Induction of vascular permeability.
•Modulation of local proteolytic factors such as collagenase and plasminogen activator.
•Induction of procoagulant activity.
•Stimulation of glucose transport into endothelial cells.

VEGF has been localised to human decidua, maternal and fetal macrophages and fetal giant cells in both first trimester and term pregnancies (20). On the fetal side of the placenta, the predominant site of expression is in the fetal macrophages within the placental villi. These cells are located adjacent to fetal capillaries, and the VEGF from these cells may be involved in the extensive angiogenesis of fetal blood vessels in the placenta during placental growth. At term, these cells are still expressing VEGF, and may then be involved in the maintenance of the endothelium or regulation of vascular permeability.

In maternal decidua, VEGF expression is located in maternal macrophages adjacent to the implantation site. In the human, flt expression at the implantation site is localised to invading extravillous trophoblast and to the trophoblastic shell surrounding the embryo. Extravillous trophoblast is therefore migrating towards the maternal decidua containing the VEGF expressing macrophages. It is probable that VEGF therefore has a role in controlling the migration and differentiation of the invading trophoblast. In support of this action, trophoblast, in a similar way to endothelial cells, is able to proliferate, migrate and produce proteases. VEGF is known to alter these proteases in endothelial cells (see Table VI) to facilitate migration during angiogenesis, and may therefore also control invasion by trophoblast.

In vitro evidence supporting this theory is shown by the stimulation of the choriocarcinoma cell line BeWo, representing a model for trophoblast, by the addition of VEGF, an effect that was specifically blocked by the addition of an anti-VEGF neutralising antibody (21).

The spatial distribution of VEGF and its receptor flt, together with the known actions of VEGF support a major role of this growth factor in normal placentation. Failure of normal expression of VEGF could therefore lead to abnormal placental development and the development of conditions such as miscarriage, pre-eclampsia, and IUGR.

Summary

There is now considerable data about the roles that many growth factors, including those whose actions were originally thought to be confined to the immune system, play in the development of the placenta. Much of the evidence of a functional role has come form studied in transgenic mice, deleted for particular cytokine genes or the corresponding receptor. In this way LIF produced by glandular epithelium has been identified as acting on pre-implantation embryos,

which bear the LIF receptor. In the absence of LIF, implantation does not occur. Other growth factors such as CSF-1, appear to play several roles CSF-1 acts directly on trophoblast to influence its growth and differentiation, however it is also important in recruitment and regulation of uterine macrophages, and the infertile *op/op* mouse indicates. More recently, we have shown that macrophages produce large quantities of VEGF, which acts on both the invading trophoblast, and to promote the angiogenesis critical to successful placentation. A summary of the interactions between the three growth factors is shown in Fig. 2.

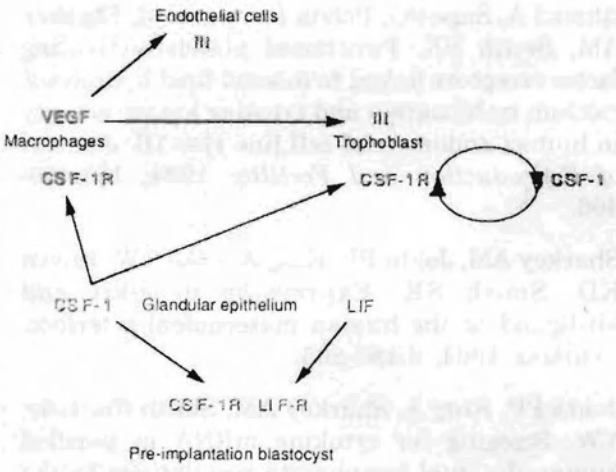

Fig. 2 : The relationship of LIF, CSF-1 and VEGF.

The clinical importance of these factors is only now beginning to be understood. Obvious areas of interest for future research and ultimately therapy, include the role of growth factors in recurrent miscarriage and infertility, and in conditions associated with inadequate placentation such as pre-eclampsia and intrauterine growth retardation. Pre-eclampsia, in particular, with its clear association with a factor released from the placenta, seems likely to benefit from this approach. Identification of this factor which appears to be responsible for maternal systemic endothelial cell dysfunction, would appears to be responsible for maternal systemic endothelial cell dysfunction, would be a major step forward in the treatment of this disease.

In other spheres growth factor therapy is already being considered, when but a few years ago this would have been unthinkable, for example, treatment for atherosclerosis with local growth factors (22). There is no doubt that the future will see an explosion of interest in these proteins.

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