

REVIEW

Molecular factors in human implantation: adhesion molecules, proteases and cytokines

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Abstract

Successful human reproduction remains an enigma, but this is slowly changing in the current era of expanding scientific knowledge. The discovery of various molecular factors such as adhesion molecules, proteases and cytokines have in recent years been at the forefront of medical research. The growing importance of immunology in particular has led to novel new immuno-modulatory therapies and increasing research into this new aspect of reproductive immunology may well prove to be the most important breakthrough in understanding the fundamentals of human reproduction.

Implantation represents the first step in the complex interactions and processes involved in foetal-maternal interaction, which continues throughout pregnancy gestation and culminates in the birth of an infant. It is therefore vital that we understand the myriad processes controlling implantation in order to build a firm foundation for exploring reproductive immunology research in the new millennium. This review brings together and presents an overview of the potential roles of currently known molecular factors such as adhesion molecules, proteases, cytokines and its interaction with the maternal immune response, incorporating the findings of previous published research performed by the author on cytokines and reproductive immunology.

IMPLANTATION

Introduction

Successful human reproduction remains an enigma, limiting our understanding of its various pathological manifestations. Progress in unravelling this mystery may come from greater understanding of the molecular factors involved in mediation of successful implantation, a crucial step in ensuring the success or failure of human reproduction. However, as ethical considerations are paramount in studies on human implantation, the currently available information have been derived mainly from *in vivo* murine or *in vitro* human models. This unavoidable limitation has contributed to the relatively slow progress of knowledge concerning human implantation.

Successful implantation is a complex process and in humans appears to be confined to an "implantation window" occurring 7 to 10 days after the mid-cycle luteinizing hormone (LH) surge, on days LH+7 to 10.¹ A multitude of progressive steps are necessary to ensure its success, the final few of which comprises apposition, adhesion and invasion.² Apposition

describes the close contact between the conceptus and endometrium prior to establishment of physical connections. When physical connections are established, the adhesion phase is entered. This proceeds to invasion when the conceptus starts to insinuate its way into the maternal endometrium. It is during this period that the semi-allograft human conceptus initially encounters maternal immune cells and coexists with them throughout its gestation, giving rise to an immunological paradox that has remained with us since its first description 45 years ago by the Nobel laureate Sir Peter Medawar.³

The recent advent of molecular biology and advances in immunology may prove to be the most significant step in understanding implantation. This has led to the discovery that three main groups of molecular factors are involved in mediation of successful implantation, comprising adhesion molecules, proteases/proteinases and cytokines. It is perhaps appropriate at this stage to summarise the major characteristics of each molecular factor group, prior to detailed discussions on their role in implantation.

Characteristics of molecular factors implicated in implantation

(i) Adhesion molecules

Adhesion molecules are proteins involved in cell surface interactions essential for normal morphogenesis and maintenance of tissue integrity in multicellular organisms. Several subgroups of adhesion molecules exist, although current evidence implicates primarily integrins and selectins in implantation.

Integrins

Integrins are membrane glycoproteins composed of two subunits (α and β) forming homologous groups with only 40-50% similarity in amino acid sequence.⁴ As integrins are cell surface proteins, they have an extracellular domain, as well as a transmembrane and cytoplasmic segment, allowing its function as a link between the cytoskeleton and extracellular matrix.^{5,6,7} The α subunit functions as the receptor binding site⁶ and is able to pair with a multitude of β subunits, creating diversity in recognition of different adhesive substrates. There are currently a total of 15 α and 8 β subunits, known as α 1-9, IIB, E, M, L, V, X and β 1-8.

Fibronectin is recognised by several integrins, comprising α 3 β 1, α 4 β 1,^{8,9} α 4 β 7,¹⁰ α 5 β 1,¹¹ α V β 1,¹² α V β 3,¹³ α V β 6¹⁴ and α V β 8.¹⁵ The integrins binding to *collagen* are α 1 β 1, α 2 β 1 and α 3 β 1,^{8,16,17} whilst *intercellular adhesion molecules* (ICAM) are recognised by α M β 2, α L β 2, *vascular cell adhesion molecules* (VCAM) by α 4 β 1, α 4 β 7¹⁴ and *laminin* primarily by α 6 β 1 as well as α 1 β 1, α 2 β 1, α 3 β 1, α 7 β 1 and α 6 β 4.¹⁸

(ii) Proteases/Proteinases

Proteases/proteinases are enzymes responsible for mediating the degradation and dissolution of extracellular matrixes. Whilst several classes of proteases/proteinases exist, the current evidence for a possible role in implantation primarily implicates serine proteases and matrix metalloproteinases.

Serine proteases

Urokinase (uPA) and tissue (tPA) plasminogen activators are serine proteases that function mainly to catalyse the conversion of plasminogen to plasmin, although they are also capable of a wide range of proteolytic activity.¹⁹ The additional properties of uPA involves cell adhesion and migration whereas tPA is involved in fibrinolysis.²⁰ In common with other enzymes,

uPA and tPA are synthesized and stored as proenzymes, and are inhibited by complexing with plasminogen activator inhibitors 1 (PAI-1) and 2 (PAI-2).²¹

Matrix metalloproteinases (MMP)

MMP form a family of homologous enzymes secreted as inactive proenzymes and incorporate a zinc atom on their active site.²² Partial hydrolysis results in loss of a propeptide and activation. Three subgroups of MMP exist, representing the only enzymes known to digest the endometrial extracellular matrix²³ and comprise gelatinases (MMP-2, MMP-9), collagenases (MMP-1, MMP-8, MMP-13) and stromelysins (MMP-3, MMP-7, MMP-10, MMP-11). The functions of each MMP are as suggested by their subgroup nomenclature, with gelatinases responsible for digestion of gelatine (denatured collagen) and type IV collagen (major constituent of basement membranes) whereas collagenases digest collagen types I-III, VII and X, the main collagen found in interstitium of extracellular matrix. In contrast, stromelysins exhibit a broad range of activity and are capable of digesting type IV collagen and gelatine (as per gelatinases) as well as type VII collagen (as per collagenases), laminin, fibronectin and proteoglycans.²

Plasmin has been shown to be a potent activator of several MMP,²⁴ together with strong evidence for cytokines.^{25,26,27,28} Once activated, the control of MMP activity is modulated by specific local tissue inhibitors, three of which has been described. Tissue inhibitors of MMP are known as TIMP-1, TIMP-2 and TIMP-3, a family of homologous, cysteine rich proteins that exert their activity by binding to the MMP. TIMP-1 and 2 appear to interact preferentially with gelatinases and stromelysins to inhibit the digestion of type IV collagen.^{29,30,31} The activators of TIMP include MMP and cytokines.³²

(iii) Cytokines

The nomenclature "Cytokine" is relatively recent,³³ although they were first recognised in 1966 by demonstration of normal macrophage migration being inhibited by materials released from sensitised lymphocytes upon exposure to antigen.³⁴ Cytokines are polypeptides involved in the control of local and systemic events of the immune response, inflammatory reactions, healing and haemopoiesis, produced mainly by immune cells although virtually every nucleated cell type in the body are capable of cytokine production. They are pleiotropic, expressing

features of 'redundancy' or 'overlap', where the effects of each cytokine are not exclusive but may be reproduced by other cytokines with overlapping functions, 'synergism/antagonism' where exposure of cells to two or more cytokines at a time may lead to different responses and 'receptor transmodulation', where a cytokine may increase or decrease the expression of receptors for another cytokine.³⁵ The number of cytokines discovered have been increasing steadily and includes interleukins (IL-1 to IL-18), tumour necrosis factors alpha and beta (TNF- α , β), colony stimulating factors (M-CSF, G-CSF, GM-CSF), transforming growth factors (TGF- α , β) and interferon gamma (IFN- γ).

The potential roles of cytokines in implantation are extensive, as cytokines have been implicated in the apposition, adhesion, early invasion and late invasion phases. The main cytokines involved comprise interleukin 1 (IL-1), leukaemia inhibitory factor (LIF), macrophage colony stimulating factor (M-CSF), as well as the T helper 1 immune response cytokines IFN- γ , IL-2, IL-12, TNF- β and T helper 2 immune response cytokines IL-4, 6, 10 and 13. It is during apposition and adhesion that IL-1, LIF and M-CSF appears to exert their effects, whereas the T helper 1 and 2 immune response cytokines potentially influence events later on at the invasion phase.

Apposition and adhesion phase of implantation

Apposition or orientation of the blastocyst within the lumen of the uterus starts on day LH+6 when the human conceptus measures 300-400 μ m in diameter and the uterine lumen is minimal due to suction of endometrial fluid by pynopods. This progresses to adhesion when the conceptus physically encounters maternal endometrium and represents the primary event in mammals initiating invasion.³⁶ Current evidence suggests that adhesion molecules and cytokines (IL-1, LIF, M-CSF) may play an important role during this period, whereas MMP may not.

The expression of α 1, α 2, α 3, α 4, α 5, α 6, α V, β 1, β 3, β 4 integrins have been demonstrated in secretory endometrium,³⁷ allowing recognition and adhesion of fibronectin (α 3 β 1, α 4 β 1, α 5 β 1, α V β 1, α V β 3), collagen (α 1 β 1, α 2 β 1, α 3 β 1) VCAM (α 4 β 1) and laminin (α 6 β 1, α 1 β 1, α 2 β 1, α 3 β 1, α 6 β 4). It is therefore interesting to note that the murine embryo expresses laminin during the two to four cell stage³⁸ and fibronectin during blastocyst formation³⁹ whereas the human embryo produces laminin at the morula stage.⁴⁰ The discovery that murine embryos are also

capable of producing α 1, α 2, α 3, α 5, α 6, α V, α 7, β 1, β 3 integrins⁴¹ further clarifies the situation, as the potential exists for the murine embryo to recognise and bind to fibronectin, collagen and laminin, therefore providing the necessary physical tools to ensure conceptus adhesion to maternal endometrium (Fig. 1).

In contrast to the physical nature of adhesion molecule involvement in apposition and adhesion during implantation, cytokines appear to exert non-physical control, by regulating cellular function including adhesion molecule expression.^{42,43} The expression of M-CSF prior to murine implantation appears to be confined to uterine epithelium and undergoes a 5 fold increase during implantation.⁴⁴ Moreover, studies on implantation utilising the osteopetrotic (op/op) mouse, a genetically modified species characterised by osteopetrosis and complete absence of M-CSF production, yielded some interesting observations. These mice were noted to be infertile, due to the inability to initiate adhesion and hence implantation, despite normal fertilisation and blastocyst formation. However, the administration of exogenous M-CSF appears to result in normal implantation.⁴⁵ Studies on humans have shown a similar pattern of M-CSF expression in the endometrium,⁴⁶ although the importance of M-CSF in human conceptus adhesion remains to be proven.

The importance of LIF was also derived from murine models, with expression detected in both the endometrium and blastocyst during the peri-implantation period.⁴⁷ This expression of endometrial LIF appears to be under maternal control as it is still expressed in artificially induced pseudo-pregnant states and absent in cases of delayed implantation by either ovariectomy or the sucking stimulus when blastocysts are floating in the endometrial cavity.⁴⁷ Moreover, deletion of the LIF gene in mice resulted in the inability of the blastocyst to implant, although fertilisation was unaffected and the blastocysts were able to be successfully implanted in surrogate female mice with LIF expression.⁴⁸ The ability to initiate implantation was also restored by exogenous administration of LIF. Human data on LIF has demonstrated its expression to be mostly confined to the epithelium fraction of the endometrium and is maximal during the implantation period.^{49,50} This expression of LIF appears to be independent of hormonal influences (oestradiol and progesterone), although other cytokines exert a modulatory effect.⁵¹ The presence of LIF receptor in human blastocysts further supports a role for

LIF in apposition or adhesion of the conceptus to maternal endometrium.⁴⁹

IL-1 comprises two distinct cytokines, IL-1 α and IL-1 β , which act on the same receptor (IL-1R). Murine endometrium is known to express IL-1 α and β , reaching a peak during the peri-implantation period, regardless of the absence or presence of the conceptus.^{52,53} More importantly, IL-1 α and β is also produced by the conceptus prior to implantation.⁵⁴ This corresponds to the presence of IL-1R within the endometrium in the peri-implantation period, which when functionally blocked by the administration of a IL-1R antagonist, prevents blastocyst adhesion to maternal endometrium and leaves it free floating within the uterine cavity.⁵⁵ The human endometrium expression of IL-1 α , β and IL-1R has been shown to have a similar pattern to murine models.^{56, 57,58} In addition, IL-1R antagonist expression has also been demonstrated within the human endometrium, with production declining as implantation approaches.⁵⁹ However, the human embryo was not consistently shown to be producing IL-1 α and β , as contradictory results were available from different authors utilising different *in vitro* models.^{60,61,62,63} The results from a recent *in vitro* study appears to clarify the observed anomalies, as differential IL-1 α and β production was observed depending on the culture media. Human embryos cultured under routine in-vitro fertilisation (IVF) conditions or co-culture with human endometrial stromal cells fail to produce IL-1 α or β , whereas co-culture with endometrial epithelial cells or endometrial epithelial cell conditioned media resulted in IL-1 α and β production.⁶⁴ These results suggest that IL-1 α and β , production by the human conceptus may be regulated by an epithelial factor yet to be identified. What it also illustrates very clearly is the technical limitations of utilising *in vitro* models to study molecular factors involved in human implantation.

Invasion phase of implantation

The process of invasion is probably the most critical step of implantation. A fine balance of regulatory activity is a prerequisite to ensure its smooth progression, as over-activity during invasion may result in pathological conditions such as placenta accreta, increta and percreta, whilst under-activity may represent the basis for miscarriage, pre-eclampsia and intrauterine growth retardation. As invasion is chronologically the longest step involved in implantation, the subsequent discussions are

divided into “early invasion”, which represent the activities up until the conceptus encounters maternal immune cells and “late invasion”, when the conceptus is in direct contact with maternal immune cells.

“Early invasion”

Early invasion is initiated when maternal tissues are digested to allow the conceptus to start burrowing into the extracellular matrix of the endometrium. The role of MMP comes to the fore during this period, as they are capable of disrupting the basement membrane, the first physical barrier to intrusion of the conceptus into the endometrium milieu. This is supported by the observation that the developing human conceptus, via its trophoblastic component, secretes a variety of MMP, especially MMP-2 and MMP-9, the gelatinases responsible for digestion of type IV collagen.^{65,66} The importance of gelatinases secretion by the human conceptus becomes clear when one realises that the main constituent of endometrium basement membrane, the first physical barrier to invasion, is type IV collagen.⁴⁰ Moreover, type IV collagen also represents a major component of the uterine extracellular matrix at the foeto-maternal interface barrier,^{67,68} the environment in which the conceptus encounters after successfully breaching the endometrium basement membrane. However, as discussed earlier, MMP are secreted as inactive pro-enzymes and therefore is dependent on other molecular factors such as plasmin and cytokines for conversion to the active state (Fig. 2).

The conversion of plasminogen to plasmin is dependent on uPA and tPA, which have been demonstrated to be produced by the developing murine blastocyst during implantation.^{69,70} In addition, under in vitro culture conditions, human trophoblast cells are known to produce uPA mainly within the first 24 hours, suggesting that it is a transient phenomenon coinciding with the moment of early invasion.⁷¹ The activity of UPA and tPA may itself be regulated by the endometrium production of its inhibitor PAI under the influence of cytokines.^{72,73} This may represent one facet of the mechanisms involved for the control of MMP activity. Once activated, MMP require strict regulation to prevent over-activity and hence over invasion, which is achieved by the presence of the MMP inhibitors, TIMP.

Cytokines are important regulators of MMP activity, as they are capable of activating MMP as well as TIMP. The regulatory role of cytokines

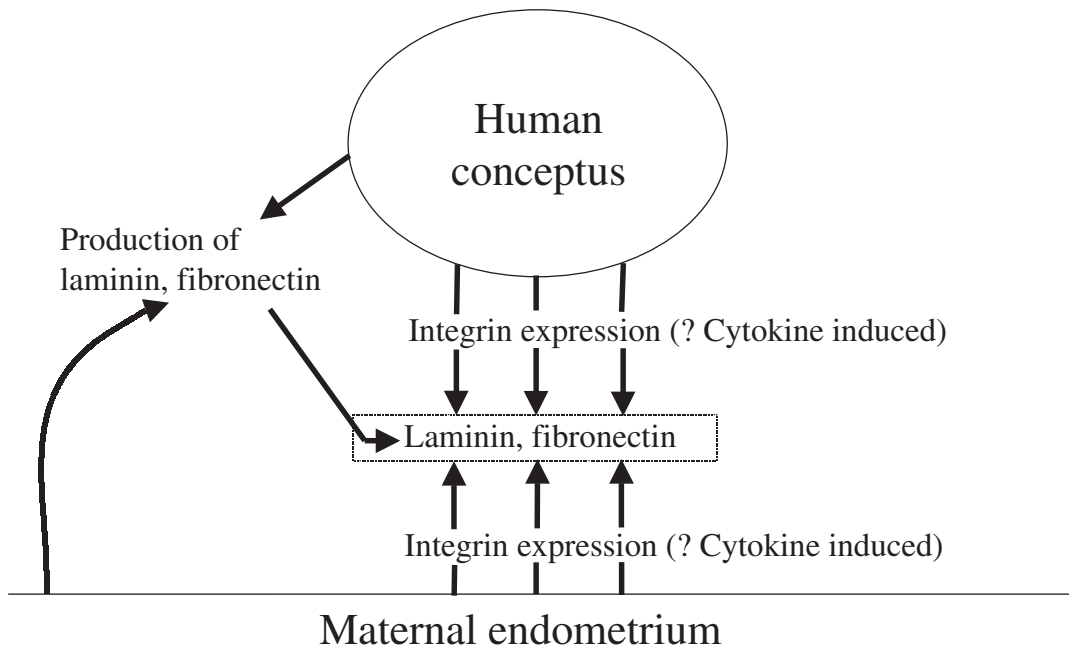


FIG. 1: Potential role of integrins and cytokines during apposition and adhesion phases of implantation

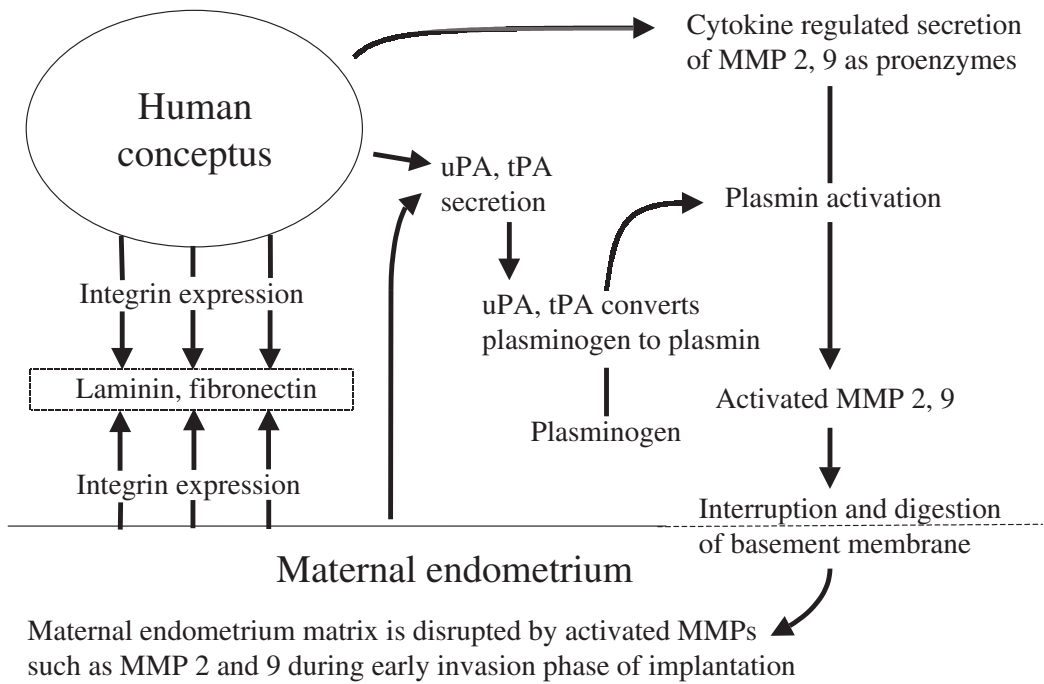


FIG. 2: Proposed interactions during early invasion phase of implantation

may be evident from the observation that TNF- α acts to increase TIMP expression in humans, therefore limiting the activity of MMP.²⁶ In addition, murine TIMP production has been shown to be upregulated by IL-1 β and IL-6,³² although the most implicated cytokine for human regulation of MMP and TIMP is TGF- β . The human endometrium is known to produce TGF- β ,⁷⁴ which possess the ability to stimulate TIMP expression in fibroblasts within the extracellular matrix, as well as inhibition of stromelysin (such as MMP-3, MMP-7, MMP-10 and MMP-11) production.²² This combination of roles seems to implicate TGF- β as a major determinant in the control of conceptus intrusion during the early stages of invasion (Fig. 3).

“Late invasion”

This represents the most crucial step of implantation, as the equilibrium achieved during this period must be maintained throughout the normal intrauterine gestation of the human conceptus. It is also during this period that the conceptus is fully exposed to the maternal environment as encountered through maternal peripheral blood circulation. Inevitably, the foeto-maternal interaction is then propelled into a new dimension, as this phase of implantation

encompasses the maiden encounter of maternal immune system cells by the conceptus. The immunological paradox that arises in normal pregnancy, whereby the semi-allograft human conceptus, which is immunologically foreign to the mother due to its paternal genetic contribution manages to evade immune rejection, was first described over 45 years ago.³ However, it is only recently that inroads have been made into understanding this enigma, with the advances in immunology and molecular biology. Despite this, human data on the events unfolding during this crucial period remain sparse because of ethical considerations. Instead, the prerogative derives from work done in murine models.

The primary function of the human immune response is to differentiate between self and non-self, leading to attack and rejection of non-self foreign bodies or antigens, known as immunogens. Evolution has resulted in the development of two distinct immune responses in vertebrates, the innate response and adaptive response, whereas invertebrates only possess an innate like immune response.^{75,76} The human innate immune response represents a more primitive, non-specific first line of defence against immunogens, mediated by neutrophils and natural killer cells.

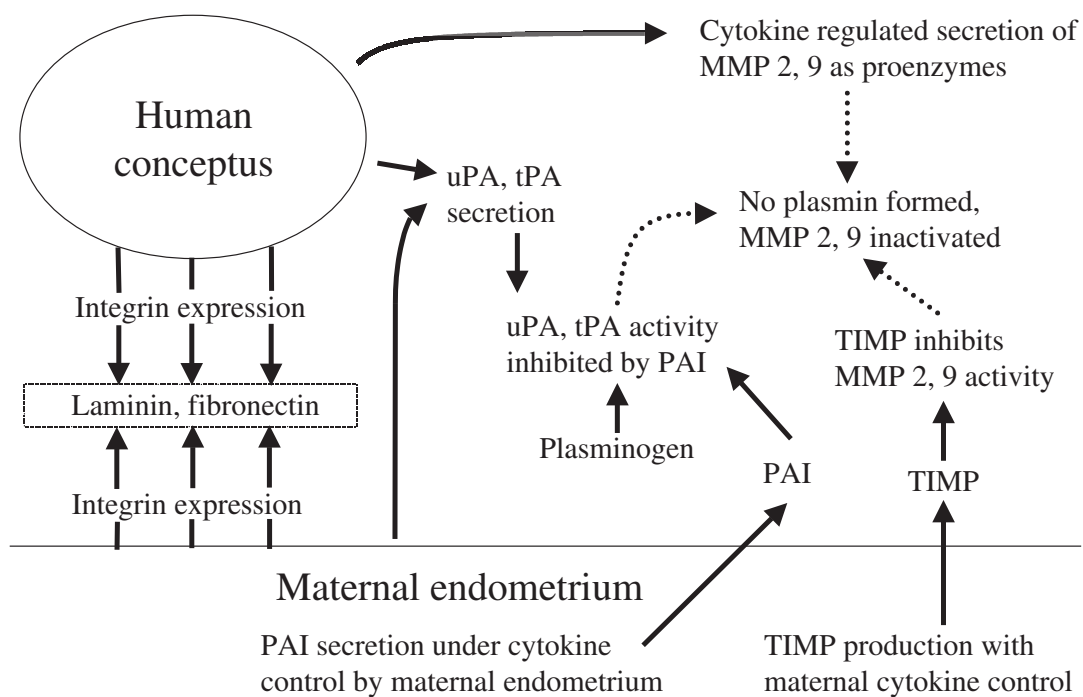


FIG. 3: Proposed control mechanisms for modulating MMP action by TIMP, PAI and cytokines

In contrast, the human adaptive immune response is a specific, targeted, second line response against particular immunogens mediated by T lymphocytes (T helper cells which express CD4, and T cytotoxic cells expressing CD8 on its cell surface), B lymphocytes and macrophages. It is initiated when an immunogen encounters antigen presenting cells (APC) such as macrophages, which process the immunogen to ensure recognition by T helper cells. These then initiate the adaptive immune response, which comprises a T helper 1 (TH-1) cytotoxic response and a T helper 2 (TH-2) humoral response depending on the type of cytokines present. The cytokines responsible for inducing a TH-1 response in humans includes IFN- γ , IL-2, IL-12, TNF- β whilst TH-2 cytokines includes IL-4, 6, 10 and 13.^{77,78} Studies on murine models have shown that the TH-1 cytokines and hence immune response are responsible for foetal rejection whereas TH-2 cytokines mediate a humoral response, which may be necessary to ensure normal reproduction.⁷⁹

However, confirmation of the *status quo* in humans is awaited, despite the available evidence showing the human endometrium to be a rich source of cytokine production and to host a variety of immune cells, which vary throughout the menstrual cycle.^{80,81,82} A different approach to that taken in murine models may be necessary to overcome the ethical constraints inherent within studies on human implantation. Progress in understanding the foeto-maternal interactions during implantation and subsequent human reproduction may derive from studies on recurrent miscarriage (RM) women, as they represent one extreme of human reproduction outcome.⁸³

The first study exploring the role of a dichotomous T helper response in human reproduction was an *in vitro* model, utilising peripheral lymphocytes extracted from unexplained recurrent miscarriage women and exposing them to choriocarcinoma cells in culture.⁸⁴ It was noted that the RM lymphocytes produced almost no TH-2 cytokines (IL-4, IL-10) but copious amounts of TH-1 cytokines (IFN- γ , IL-2, TNF- β), whereas the converse was true for controls consisting of men and fertile women. However, as the endometrial and peripheral immune cell populations may be inherently different,⁸² a more reserved interpretation of the results is necessary. An attempt to overcome this limitation was made in a recent study, which utilised immune cells

extracted from the decidua of four unexplained RM women. The small sample source necessitated *in vitro* expansion of the immune cells, prior to chemical rather than biological stimulation to determine the profile of cytokine production.⁸⁵ In contrast to the previous study, no difference in TH-1 cytokine (IFN- γ , TNF- β) production by RM women was observed, although the TH-2 cytokine (LIF, IL-4, IL-10) production was lower compared to decidual lymphocytes from women undergoing termination of pregnancy. An additional finding relating to progesterone was that TH-2 cytokine expression was induced *in vitro*, but only at non-physiological levels exceeding 4,770nmol/L.

Clarification of the conflicting *in vitro* human results may be provided by our studies analysing the *in vivo* profile of TH-1 and TH-2 cytokine expression within peri-implantation endometrium of 10 normal and 25 RM women. Due to ethical considerations, the endometrial specimens were collected during perceived implantation (days LH+7 to LH+10) of a non-conception cycle, then analysed by reverse transcription polymerase chain reaction (RT-PCR) and enzyme linked immuno-sorbent assay (ELISA) for the respective presence of mRNA and protein secretion of TH-1 (IFN- γ , IL-2, IL-12, TNF- β) and TH-2 cytokines (IL-4, 6, 10, 13). In addition, the influence of systemic hormones (FSH, LH, oestradiol, progesterone, testosterone and DHEAs) on TH-1 and TH-2 cytokine expression *in vivo* was also investigated.

The results yielded some interesting observations. Normal women, defined as those with no previous miscarriage history and at least one successful vaginal livebirth delivery, exhibited a major paucity of TH-1 cytokine expression,⁸⁶ especially of IL-12, the key cytokine involved in initiation of a TH-1 response⁸⁷ and IFN- γ , the corresponding cytokine involved in perpetuation of a TH-1 response.⁷⁸ The converse was observed for the TH-2 cytokines IL-4, critical for the establishment of a TH-2 response^{88,89} and IL-6, which perpetuates the TH-2 response. However, there was no dominant expression of the cytokines implicated in antibody production, IL-10 and IL-13,⁹⁰ perhaps explaining why studies in normal fertile women consistently fail to detect allograft protective "blocking antibodies"^{91,92} (Figure 4).

The opposite situation was observed in RM women, results of which were similar regardless of the underlying RM aetiology as currently recognised.⁹³ This represents a potentially

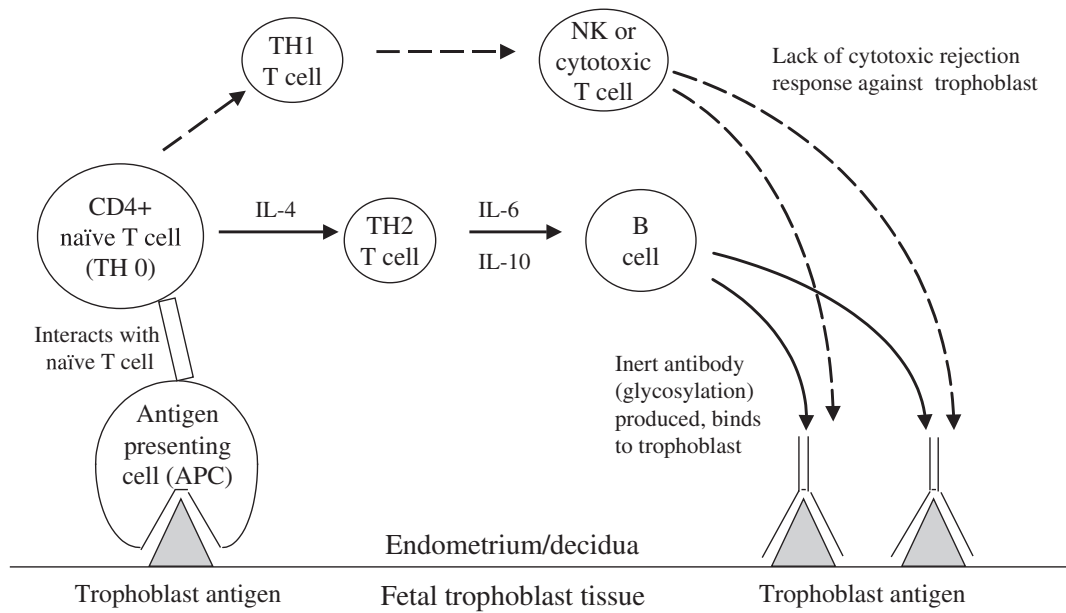


FIG. 4: Proposed maternal immune response during late invasion phase of normal implantation.

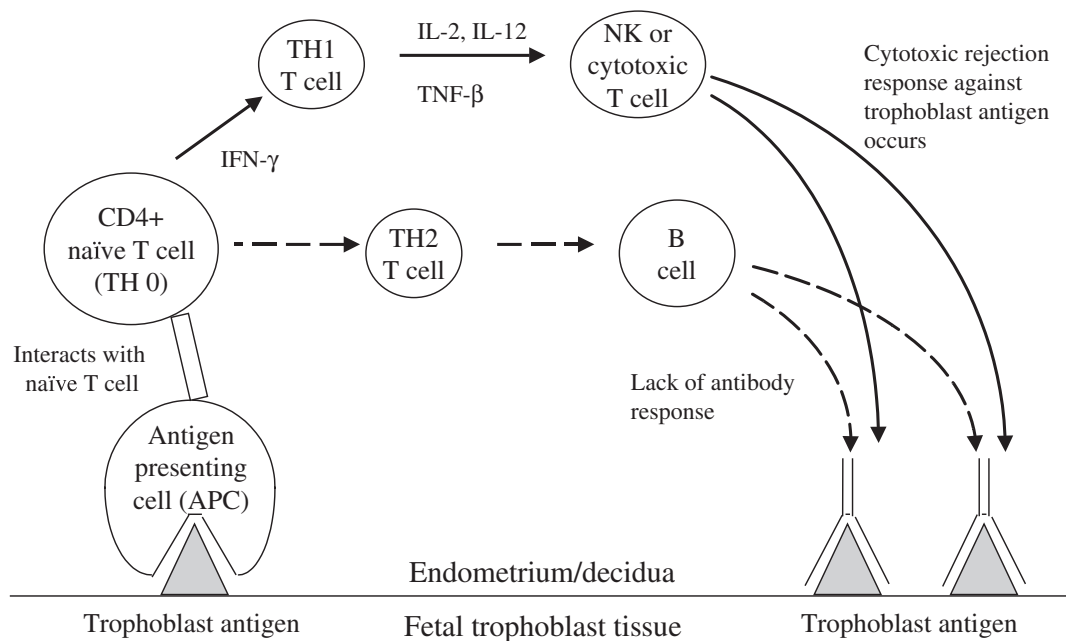


FIG. 5: Proposed maternal immune response during late invasion phase of recent miscarriage implantation.

important point as it suggests that the findings are not confined to unexplained RM and may be applicable to other pathological manifestations of human reproduction. It is therefore noteworthy that all the TH-1 cytokines studied (IFN- γ , IL-2, IL-12, TNF- β) had significantly greater expression in RM women compared to controls. The resulting environment would not be conducive to maternal immune tolerance of the human conceptus, indeed, it would facilitate a TH-1 rejection response, manifest clinically by the repeated miscarriages. Indeed, analysis of the TH-2 cytokine results supported this as a paucity of IL-4 and IL-6 secretion was observed, contributing further to the TH-1 dominance. This may be interpreted as supporting the *in vivo* presence of a predominant TH-2 response mediating successful implantation and a predominant TH-1 response mediating rejection of the human conceptus (Figure 5).

It is tempting to postulate a potential interaction between the endocrine and immune systems via progesterone induced modulation of cytokine production, as this would correlate with the various observations showing the importance of progesterone in human reproduction. The discovery that progesterone was indeed able to induce TH-2 cytokine (IL-4) production *in vitro* appears to provide the missing link, but this effect was only observed at progesterone levels exceeding 4,770nmol/L.^{85, 94} However, as the serum progesterone level in normal pregnancy ranges from 100 to 500nmol/L,⁹⁵ it becomes evident that the *in vitro* and *in vivo* differences are of an order of magnitude, tempering the initial excitement. Furthermore, no correlation was evident *in vivo* between serum hormone levels (FSH, LH, oestradiol, progesterone, testosterone, DHEAs) and endometrial TH-1, TH-2 cytokine expression from our study.⁹³

Summary of current knowledge on implantation

Slowly but surely, inroads are being made into understanding the enigma of human reproduction through our greater understanding of the molecular events involved in mediating successful implantation. The importance of molecular factors is only just beginning to be understood, with cytokines forming the common thread throughout the processes encountered during implantation. Cytokines are regulators of cellular function and have been shown to control the production and activities of adhesion molecules, proteases/proteinases as well as

having a pivotal role in initiating and perpetuating the different arms of the maternal immune response. The role of adhesion molecules appears to be maximal during the apposition and adhesion phases of implantation, by forming the necessary physical link, whilst proteases/proteinases appear to be necessary to initiate the invasion phase of implantation by disrupting the basement membrane through enzymatic action. This preliminary understanding concerning the myriad events associated with implantation represents the foundation to build on, as the major limiting factor affecting current attempts to improve human reproduction outcome is unsuccessful implantation. The implantation failure rate in normal fertile women has been demonstrated to be 17% to 22%,^{96,97} whereas infertile women undergoing assisted conception have an implantation failure rate of 85%.⁹⁸ It is evident that successful human reproduction remains an inefficient process, whilst the currently available treatments appear to have a low therapeutic value.

Future directions for implantation research in the new millennium

Why is it necessary to invest in research aimed at unravelling the mysteries of implantation when a variety of clinical treatments is currently available for the pathological manifestations of deficient implantation such as pre-eclampsia, miscarriage and intrauterine growth retardation? The answer becomes clear when the underlying basis for the available treatments is explored, as the current therapies can only delay or minimise the after-effects but not reverse the natural progression of pre-eclampsia, miscarriage and intrauterine growth retardation. If the events and control mechanisms involved in implantation are understood, it would then be possible to modulate them. This would result in more efficient treatments and prevention of embryo wastage as currently experienced during *in vitro* fertilisation, leading to an improved reproductive outlook for unexplained infertility, RM and other clinical manifestations of deficient implantation.

A multitude of future research pathways is to be traversed for this to become reality in the new millennium. These include the development of a new *in vitro* matrix system which replicates as much as possible the complex endometrial milieu and hence overcome the current limitations of Matrigel (Collaborative Research Inc, Bedford, Massachusetts) in studying implantation;^{99,100} a novel method of delivering cytokines to the

uterine cavity that improves on the current liposome technology;¹⁰¹ continuation of implantation research utilising murine models; the advent of cytokine immuno-modulation to alter maternal T helper immune response as tried successfully in animal models to prevent repeated miscarriages^{102,103} and even gene therapy, to selectively inhibit or induce cytokine production *in vivo* to achieve an environment conducive to successful implantation.

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