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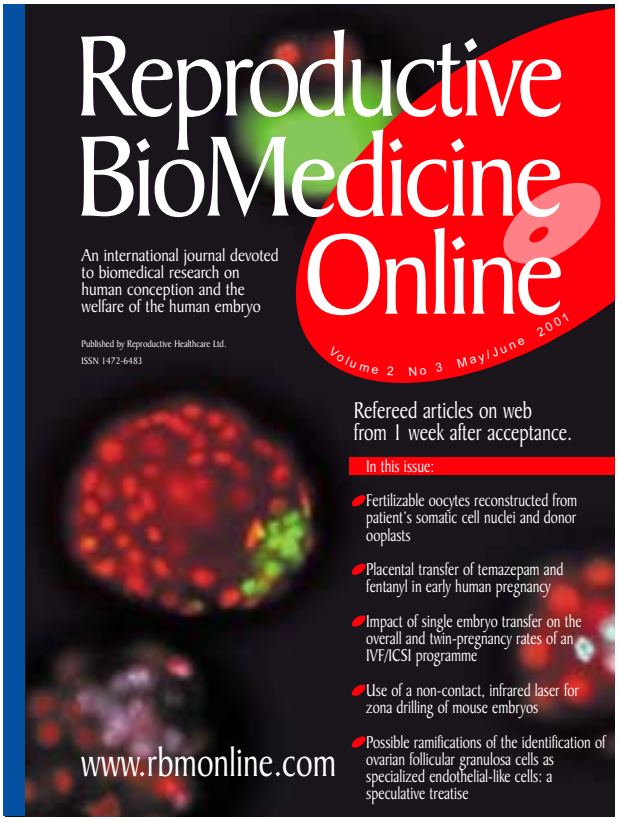


# New insights into maternal-fetal interactions at implantation

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Articles

# New insights into maternal-fetal interactions at implantation*

Dr Chaouat switched from T cell tolerance to materno–fetal relationships (initially enhancing antibodies and suppressor T cells). He moved to placental suppressor anergising T lymphocytes. Other suppressors were studied with Drs Wegmann, Clark and Szekeres-Bartho. He focussed on utero–placental cytokines, using the CBA x DBA/2 murine abortion model ("immunotrophic" cytokines, role of Th2 cytokines, placental tau interferons with J Martal). Current topics are cytokines in implantation, placental suppressor factor targets, mechanisms of TNF, stress/RU486-induced abortions, parturition and vertical transmission of HIV (trophoblast selection of viral variants, role of local cytokines/β chemokines).

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## Abstract

The immunotrophic theory was enunciated by Tom Wegmann. Since then, the involvement of cytokines in implantation and materno–fetal tolerance has emerged as a central topic in reproductive immunology. This brief survey covers the historical background leading to the specification of the crucial role of cytokines at the feto–maternal interface, and the present known patterns of their function. Focus is addressed to the most recent concept, e.g. pregnancy as a Th2 phenomenon (the immune response of the mother is biased towards the production of anti inflammatory cytokines, amongst them IL-10 which suppress inflammatory responses). A brief description of the role of inflammatory cytokines in implantation is presented to explain why it does not fit into such a scheme, and other recent data, for example on γ interferon, also fails to accord with the Th2 phenomenon.

***Keywords***: *cytokines, immunology, implantation, materno–fetal interactions, pregnancy*

## Introduction

The field of reproductive immunology, especially its sub-specialty dealing with immunology of pregnancy, passed through a profound transition when Medawar discovered "the riddle of the fetal allograft" (Medawar, 1953). He realized as early as 1953 that according to the then recently discovered laws of transplantation that the maternal immune system should reject the fetus. This paradox became even more apparent when Wooodruff reported that ectopic grafts of fetal tissues could be rejected whilst the fetus itself continued invading the uterus and, later on, went on thriving (Woodruff, 1958).

The consequences of such an experiment were not necessarily fully understood at the time. One potential exception to this judgement perhaps included David Clark in his original papers on local suppression in the uterine lymph nodes draining lymph nodes (Clark and McDermott, 1978; Clark *et al*., 1980). The experiment implied a dissociation between local acceptance of the fetus and systemic immune responses, since the latter could be triggered without compromising local acceptance of the fetus, as also seen by Mitchison(1953) and Lanman *et al*. (1962).

This perspective was somehow neglected, as were the aforementioned pre-immunization experiments. Indeed, investigators then for years centred on the acceptance of the fetus by the mother as a systemic T cell tolerance of the fetus. It was then discovered that in pre-immunized mice, circulating anti-paternal cytotoxic T lymphocytes had become sensitized against paternal MHC antigens. They were able to reject a paternal-strain tumour allograft without harming the conceptus. This led to our own challenge to the concept of systemic tolerance (Monnot and Chaouat, 1984). A mandatory the role of enhancing antibodies and suppressor T cells was dismissed even though we had also played a significant part in their discovery (Voisin and Chaouat, 1974; Chaouat and Voisin, 1979).

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Recent developments have made it even more necessary to reconsider such a proposal in view of the role of cytokines at the feto–maternal interface. In fact, recent developments seriously question the allograft oncept as presenting an strong reductionist perspective which could be seriously misleading. Indeed, the feto–maternal relationship might be more akin for some to a host–tumour or a host–parasite relationship than to an host–graft situation (Loke and King, 1995).

Another difficulty challenging the concept of maternal immune tolerance came from studies dealing with implantation. The role of colony stimulating factor 1 (CSF-1) was identified by Bartocci *et al.* (1986). It also became apparent that in mice implantation required an inflammatory-like local reaction elicited post-coitum by components present in seminal fluids. Pioneer studies included those by Wood and co-workers and MacMaster and co-workers in rodents (Sanford *et al*., 1992; MacMaster *et al*., 1992), following our own studies at the cellular level (Noun *et al*., 1989; Kachkache *et al*., 1991). In humans, the role of interleukin 1 (IL-1) was similarly pointed out by Simon *et al*. (1994a, b). Albeit, the initial experiments of this group in mice (1994) are somehow controversial since IL-1 knock-out mice have a perfectly normal pregnancy.

These interpretations must lead us to reconsider the status of the feto–maternal interface. This is especially needed since the cytokine profiles observed in the pre- and peri-implantation periods are quite opposed to those observed once pregnancy is established. They are indeed incompatible with the concept of allopregnancy viewed only as a maternal immunological tolerance of the fetus.

Finally, the most recent development is the correct understanding of the role of the innate immune system. The importance of the role of so-called natural killer cells (NK) has been recognized as pivotal in the feto–maternal relationship (Guimond *et al*., 1998). Last but not least, Loke and King (1996) became pioneers in this reorientation, with their complete reappreciation of the utility of animal models. This is due in large part to the peculiar antigenic status of the placenta in primates. The discovery of human leikocyte antigen G (HLA-G) at the feto–maternal interface by Ellis (1990), and its NK regulating function, has re-emphasized the immunoregulatory role of HLA-G in primates since the original experiments of Kovatts *et al*. (1991).

## Recent concepts

The field of study has thus moved from a vision of the placenta being mostly tolerated because of enhancing antibodies to the discovery of the role of a “sea of cytokines” in which the embryo is literally immersed. It should be stated that this new perspective owes a great deal to two seminal proposals formulated by the late Tom Wegmann. These are: (i) the immunotrophic theory (Wegmann, 1984), the independent and almost parrallel dicovery of the role of CSF-1 (Bartocci *et al*., 1986) and the subsequent elucidation of the role of IL-3 and granulocyte macrophage (GM) CSF in pregnancy (Athanassakis *et al*., 1987; Chaouat *et al*., 1990). These discoveries proved how correct this theory was at the time; (ii) the more recent proposal that “allopregnancy is a Th2 phenomenon” (Wegmann *et al*., 1993) was subsequently demonstrated in mice (Chaouat *et al*., 1995; Krishnan *et al*., 1996a,b) and subsequently in humans (Hill, 1995; Piccinni and Romagnani, 1996; Raghupathy *et al*., 1999).

Those proposals have been immensely stimulating. Yet they did not fully forecast the role of cytokines in implantation. Indeed, the assertion that pregnancy is a Th2 phenomenon is incompatible with the discovery that a pro-inflammatory like environment is involved in implantation. At least two cytokines in that group have been found to be mandatory for implantation in mice, i.e. leukaemia inhibitory factor (LIF) (Stewart *et al*., 1992) and IL-11 (Robb *et al*., 1998). For LIF, Stewart *et al*. (1992) elegantly demonstrated that the maternal production of LIF is mandatory for successful implantation. LIF-deficient mice, obtained by gene knockout, are fertile and the embryos are normal. But they do not implant at all. Successful implantation of embryos in human interleukin for DA cells (HILDA)-LIF deficient mice can be induced via supplementation with recombinant HILDA LIF using an osmotic pump (Stewart *et al*., 1992). Evidence was also obtained that defects in uterus-specific LIF production could be involved in human sterility (Delage *et al*., 1995), and this conclusion has been confirmed by other investigators (Laird *et al*., 1997).

It now appears, however, that this question is more complex than originally thought. First, our studies have shown how an excess of LIF in uterine flushings probably arises from the cervix rather than from the intrauterine epithelium. Amounts are associated with high levels in such flushings of tumour necrosis factor a (TNFa), and can cause a deficient implantation. Correcting TNF levels leads to a lowering of the LIF levels in the flushings. Subsequently, this return to normal is often associated with a successful pregnancy (Ledée *et al*, 2001). It must be stressed that this is still an open study.

A second degree of complexity is presented by the recent discovery of the existence of LIF isoforms, with a related tissue-specific regulation of LIF isoform production. Some isoforms are much more active than others (Giess *et al*., 1999). Also, pinpoint LIF mutations have recently been described (Haines *et al*., 1999). Thus, some women might have quantitatively normal LIF levels in their uterus, but a qualitatively deficient isoform expression leading to excess local production of the hypoactive molecule. Other women with low LIF production might still be fertile because they predominantly express the most active moiety.

We have now related the effects of LIF to implantation. In fact, local, peri-implantation, inflammatory responses with optimal expression of adhesion molecule in placental trophoblast and uterine decidual cells are necessary for implantation to occur. This does not mean that LIF acts directly on such adhesion molecules, and therefore on embryo attachment; indeed, contradictory data have been presented.

Early and first trimester trophoblast express both laminin and fibronectin receptors, and specifically the $\alpha_1\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_{1vd}$, $\alpha_6\beta_4$ integrin heterodimers. Their receptor molecules (laminin and fibronectin) are particularly abundant in the peri-implantation as well as first-trimester uterus. Trophoblast cell themselves down-regulate the expression of the $\beta_4$ integrin and up-regulate the $\beta_1$, $\alpha_5$, and $\alpha_1$ subunits during the invasion process (Jokhi, 1994; Jokhi *et al*., 1997; Aplin, 1996).

Quantitative LIF deficiency in a group of infertile women has been linked with abnormal low levels of $\alpha_5\beta_1$ integrins in the preimplantation human uterus (Lapprée Delage *et al*., unpublished observations).

The need for such an inflammatory cytokine as LIF is opposed to the concept of pregnancy being solely dominated by Th2. It already points to the existence of several 'windows' with selected cytokine profiles. Yet, the Th1/Th2 paradigm is still stated as dominant by most investigators studying established pregnancy. Grossly speaking, the established pregnancy is still characterized by low levels of the Th1 cytokines (TNF, γIFN ) which are known to be abortifacient in a variety of animal models. They are likely to be so in humans also (Chaouat *et al*., 1990, 1995; Tangri and Raghupathy, 1993; Hill, 1995; Krishnan *et al*., 1996a, b; Raghupathy *et al*., 1999). Conversely, Th2 cytokines, amongst them IL-10, appear to play a protective role in pregnancy (Chaouat *et al*., 1995; Piccinni and Romagnani, 1996; Raghupathy *et al*., 1999).

But both the "immunotrophic" (Wegmann, 1984) and even the Th1 /Th2 (Wegmann *et al*., 1993) paradigms (**Figure 1**) were enunciated at a time when confrontation or dialogue with the mother was seen to be mediated mostly by T cells. However, this scheme soon appeared to represent a simplification since the vast majority of interleukins and "immune cytokines" appeared to be synthethized by cells of non-immune origin in the reproductive tract. An example of this is indeed the case for most IL-10 secretion (Roth *et al*., 1996; Chaouat *et al*., 1999). Furthermore, abortion in mice appeared to be dependent on NK rather than on classical αβ T cells (Baines and Fougerolles, 1988; Kinsky *et al*., 1990; Baines and Gendron, 1993). But, surprisingly, an involvement of NK cells was also demonstrated in the correction or prevention of abortion in the murine CBA x DBA/2 model. It could be shown that correcting low IL-10 levels in the placentae of alloimmunized CBA/J mice was dependent upon NK, but not on T cells (Chaouat *et al*., 1998). This was associated with the prevention of fetal resorptions, demonstrating for the first time a dual involvement of NK cells.

Subsequently, an important though still controversial step was the discovery of the role of γδ T cells in the decidua (Arck *et al*., 1997). But the key experiments were those leading to the discovery by Croy and colleagues that placentae of NK deficient mice were grossly hypotrophic, which led to premature fetal death (Guimond *et al*., 1998). Recent

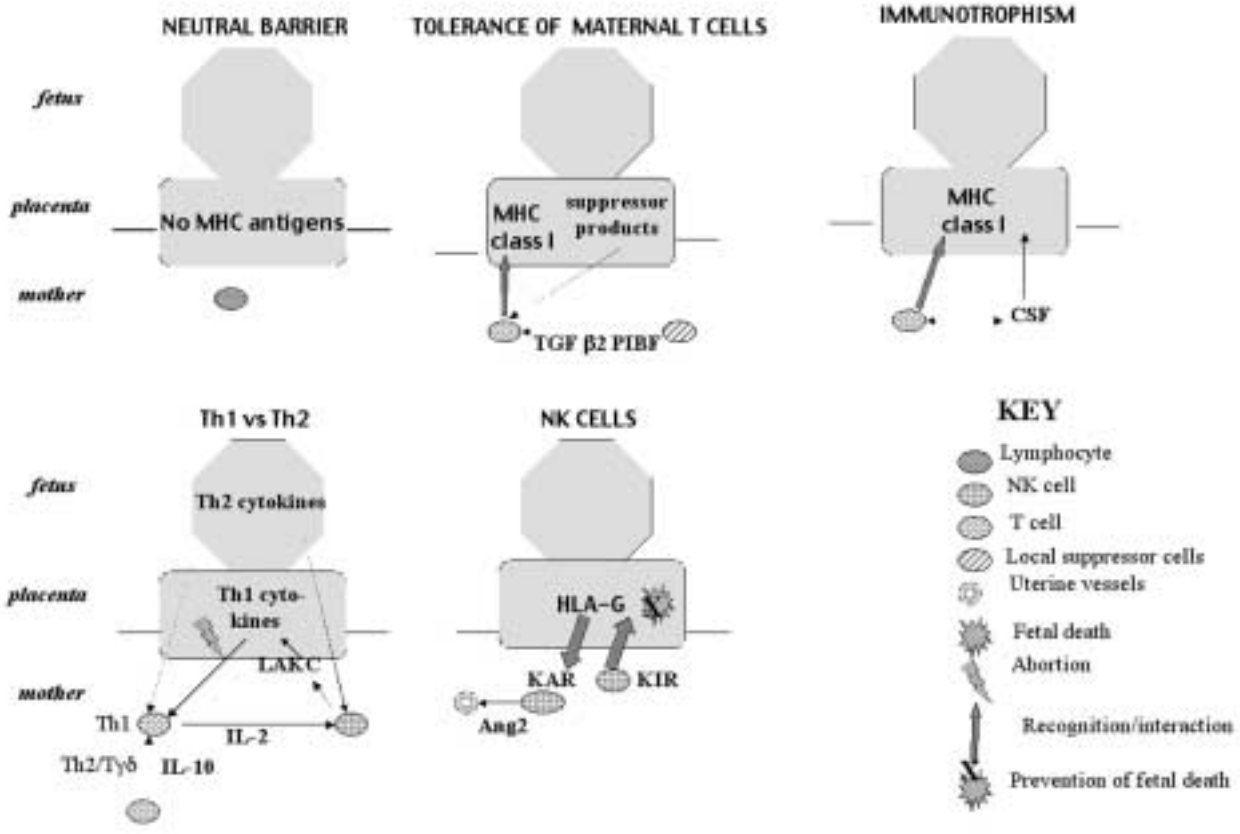


**Figure 1**. Diagram illustrating five successive mechanisms proposed for the immuno protection of the materno–fetal barrier. The light oval represents the fetus, the smaller dark oval the placenta, and the box is the uterus. The proposals describe the neutral barrier hypothesis: the placenta and decidua cause maternal T cells to be locally tolerated; immunotrophism, Th1 versus Th2, and NK cells are also depicted. Some of the pros and cons for these mechanisms are shown in **Table 1**. See footnote to Table 1 for abbreviations.

**Table 1**. Successive theories on the immunoprotection of the fetus.

| Theory and details | Comment |
|---|---|
| *The neutral barrier (1960s)*<br><br>Placenta is a neutral shield lacking MHC antigens between mother and fetus, itself anchored in the uterus – an immunologically privileged site | No placental MHC antigens doubtful. Cons: class I MHC antigens exist on trophoblast, uterus has antigen-presenting capacity, allo-antibodies are elicited in pregnancy and bind to trophoblast paternal class I MHC<br>itself anchored in the uterus – an immunologically privileged site |
| *Placenta controls maternal systemic responses (1970s) (not shown in Figure 1)*<br><br>Placenta regulates allo-antibody formation and 'deviates' maternal immune reaction to elicit 'facilitating-enhancing' antibodies and suppressor T and non-T cells at the systemic level and in draining lymph nodes, and/or placenta secretes non-specific 'blocking factors' | MHC antigens class I found in mice. Cons: pregnancy normal in ag-globulin women and in Ts deprived animals. Also pregnancy normal and placentae larger in females preimmunized against paternal allo-antigens which reject paternal skin grafts in a secondary fashion |
| *Immunotrophism*<br><br>Maternal T cells recognize placental MHC class I and secrete cytokines (mostly CSF) which act as growth factors on trophoblast | T cells not completely tolerated and not solely a threat to the fetus. Cons : still valid, perspective incomplete |
| *Placenta and decidua cause maternal T cells to be locally tolerated*<br><br>Placenta secretes soluble factors and/or soluble MHC class I antigens that energize or induce clonal deletion of T cells. Cells secreting TGFβ2 and T cells producing PIBF complete this suppression at interface. T cells are main threat to fetus | Cons: still valid, but perspective incomplete |
| *Th1 versus Th2*<br><br>Th1 cells (or activated NK cells) are abortifacient. Th2 cells, and placental (mostly) and decidual cells (mostly gd) secrete Th2 cytokines (IL-10, etc) which control Th1 responses | The concept has evolved further by considering that Th2 and Th1 cytokines are secreted mostly by non-immune cells in the reproductive tract |
| *The NK cells*<br><br>These cells can damage trophoblast and secrete Th1 products causing fetal death unless suppressed by HLA-G interaction with KIR. But interaction with KAR stimulates production of trophic cytokines and angiogenic factors | The natural immune response is an integral and prominent part of the materno–fetal relationship |

CSF = colony stimulating factors; HLA = human leukocyte antigen; Ig = immunoglobulin; IL = interleukin; KAR = killer activating receptor; KIR = killer inhibiting receptor; MHC = major histocompatibility complex; NK = natural killer; PIBF = progesterone induced blocking factor; TGF = transforming growth factor; Th1/Th2 = T helper cell 1/2; Ts = suppressor T cells.

reconstitution experiments show how this defect can be corrected by reconstituting the NK compartment (Guimond *et al*., 1998). The data showing that the production of immunotrophic cytokines might be, in fact, under the direct control of NK cells suggest that 'allorecognition' of pregnancy is in fact exerted by NK rather than by T cells. Coupled data from the same investigators shows how interferon γ is necessary for the initiation of proper uterine vascularization (Ashkar *et al*., 2000; Croy *et al*., 2000). It is not solely a 'bad guy' cytokine.

## Summary

The bulk of the aforementioned data should lead to a reappreciationand reclassification of the role of cytokines. They have had an hitherto poorly suspected role. Nevertheless, Loke and King (1996) forecast that HLA-G exerted control over the production of immunotrophic cytokines. It was not solely the local defusing agent of NK-mediated cytolytic functions. Data already exist in this respect (Maejima *et al*., 1997).

The Th1 Th2 paradigm is further complicated by our own recent studies (Chaouat *et al*., 2000), as summarized elsewhere (Chaouat *et al*., unpublished). We have undertaken a systematic study of the expression of IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17 and IL-18 in the uterus, peri-implantation embryo, and later in decidual and placental tissues throughout pregnancy. All these cytokines are found at the feto–maternal interface.

The complex pattern of expression observed is difficult to reconcile with a pure Th1/Th2 paradigm, and this impression is further strengthened by the additional complexity unravelled in the placenta and decidua at later stages of pregnancy. **Figure 1** shows a diagrammatic representation of various mechanisms proposed.

## Addendum

As stated elsewhere, the cytokine network assumes various functions in an intricate and redundant fashion (Callard *et al*., 1999). The network is certainly governed by chaos (in a mathematical sense) and redundancy makes the dissection extremely difficult using a reductionist approach, as shown by the quasi-general failure to predict the outcome of a knockout experiment. Hence, very few gene defects are likely to give an all out problem, as nevertheless seen in a few cases, e.g. LIF, IL-11. An apparently similar defect, e.g.functional or clonal NK deficiency, can lead to two different results. Some NK cell-deficient mice, where the NK are probably deleted, such as theTgE26 mice, have as a consequence repeated pregnancy failure, (Guimond *et al* 1998) , while mice which have a functional NK deficiency, such as the IL-2 common receptor γ chain deficient mice, have a normal pregnancy (Miyazaki *et al* 2000).Such studies exemplify the need for complete analysis of the discrete molecular entity that is affected. Redundancy of the network may call for sophisticated mathematical models as excellently discussed by Callard *et al*. (1999) and elsewhere.

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