New insights into maternal-fetal interactions at implantation

Gérard Chaouat1,5, Sandrine Zourbas1,2, Sasa Ostojic1,3, Geneviève Lapprée-Delage1, Sylvie Dubanchet1, Natalie Ledée1,4, Jacques Martal1

1U 131 INSERM, Équipe cytokines et relation materno foetale, Hôpital Antoine Béclère. 92141 Clamart, France
2INRA, Endocrinologie de l’embryon. 78350 Jouy en Josas Cedex
3Current address: University of Rijeka, Faculty of Medicine, Department of Biology, Brace Branchetta 22, 51000 Rijeka, Croatia
4Service de Gynécologie Obstétrique, Hôpital Antoine Béclère. 92141. Clamart. France
5Correspondence: Tel : +33 1 45 37 44 50; e-mail: chaouatg@worldnet.fr
Articles

New insights into maternal-fetal interactions at implantation*

Dr Gérard Chaouat

Gérard Chaouat1,5, Sandrine Zourbas1,2, Sasa Ostojic1,3, Geneviève Laprée-Delage1, Sylvie Dubanchet1, Natalie Ledée1,4, Jacques Martal1

1U 131 INSERM, Equipe cytokines et relation materno fœtale, Hôpital Antoine Béclère. 92141Clamart, France
2INRA, Endocrinologie de l'embryon. 78350 Jouy en Josas Cedex
3Current address: University of Rijeka, Faculty of Medicine, Department of Biology, Brace Branchetta 22, 51000 Rijeka, Croatia
4Service de Gynécologie Obstétrique, Hôpital Antoine Béclère. 92141. Clamart. France
5Correspondence: Tel : +33 1 45 37 44 50; e-mail: chaouatg@worldnet.fr

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-speciality 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 Woodruff 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 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).

*This manuscript is based on an oral presentation at the meeting organized in New Delhi in November 2000 by Dr Satish K. Gupta
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 concept 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 knockout 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 reapprreciation 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 discovery of human leikocyte antigen 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 parallel discovery 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 (Athanasakis 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 intraterine 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 $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_4\beta_1$, $\alpha_4\beta_1$ 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 $\beta_1$ integrin and up-regulate the $\beta_1$, $\alpha_5$, and $\alpha_4$ 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, $\gamma$IFN) 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 Raghuopathy, 1993; Hill, 1995; Krishnan et al., 1996a, b; Raghuopathy 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; Raghuopathy 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 $\alpha\beta$ T cells (Baines and Fougérolles, 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 $\gamma\delta$ 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.**

<table>
<thead>
<tr>
<th>Theory and details</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The neutral barrier (1960s)</strong></td>
<td>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 itself anchored in the uterus – an immunologically privileged site</td>
</tr>
<tr>
<td>Placenta is a neutral shield lacking MHC antigens between mother and fetus, itself anchored in the uterus – an immunologically privileged site</td>
<td></td>
</tr>
<tr>
<td><strong>Placenta controls maternal systemic responses (1970s)</strong> (not shown in Figure 1)</td>
<td>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</td>
</tr>
<tr>
<td>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’</td>
<td></td>
</tr>
<tr>
<td><strong>Immunotrophism</strong></td>
<td>T cells not completely tolerated and not solely a threat to the fetus. Cons: still valid, perspective incomplete</td>
</tr>
<tr>
<td>Maternal T cells recognize placental MHC class I and secrete cytokines (mostly CSF) which act as growth factors on trophoblast</td>
<td>Cons: still valid, but perspective incomplete</td>
</tr>
<tr>
<td><strong>Placenta and decidua cause maternal T cells to be locally tolerated</strong></td>
<td>Cons: still valid, but perspective incomplete</td>
</tr>
<tr>
<td>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</td>
<td></td>
</tr>
<tr>
<td><strong>Th1 versus Th2</strong></td>
<td>The concept has evolved further by considering that Th2 and Th1 cytokines are secreted mostly by non-immune cells in the reproductive tract</td>
</tr>
<tr>
<td>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</td>
<td></td>
</tr>
<tr>
<td><strong>The NK cells</strong></td>
<td>The natural immune response is an integral and prominent part of the materno–fetal relationship</td>
</tr>
<tr>
<td>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</td>
<td></td>
</tr>
</tbody>
</table>

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 (Guimonet al., 1998). The data showing that the production of immunotrophic cytokines might be, in fact, under the direct control of NK cells suggest that ‘allorrecognition’ 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 (Ashkaret al., 2000; Croyet al., 2000). It is not solely a ‘bad guy’ cytokine.

Summary

The bulk of the aforementioned data should lead to a reappraisal and 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 cytokolytic functions. Data already exist in this respect (Maejimaet 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 plaenta 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 of a 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.

Acknowledgements

This review is dedicated to the memory of my ever challenging friend, the late and missed Tom Wegmann.

References


Chaouat G, Assal Meliani A, Martal J et al. 1995 IL-10 prevents inflammatory cytokine-mediated fetal death and is inducible by the T cell receptor. Journal of Immunology 152, 2411–2420.


