One of the important discoveries in reproduction is that the reproductive process uses for its own purpose lymphokines and cytokines which were initially defined as being of immunologic nature, first traced within the immune system. This is not very surprising, since most of these molecules are more signaling factors and communication molecules between cells of various lineages than molecules strictly restricted to the immune system, such as immunoglobulins (Ig) and T-cell receptors. The field has now evolved from the idea of a host-graft relationship to the concept of an integrated dialogue between the mother and the conceptus, more akin, for some, to a host-tumour relationship. Yet, in fact, the paradigm of acceptance and/or rejection of the foetus by its mother is still heavily embedded in the thinking of many reproductive immunologists.

This is not surprising, since the trend of research that led to modern reproductive immunology was initiated by Sir Peter Medawar’s disquisition on "the riddle of the foetal allograft." It is therefore not all disconcerting that, in 1997, one of Medawar’s colleagues, Leslie Brent, in his recent book A History of Transplantation Immunology, still depicts the materno-fetal relationship as “Nature’s (almost) perfect allograft,” nor that pioneering work in the field is due to another collaborator of Leslie Brent and Peter Medawar, Rupert E.Billingham.3

However, the paradigms have evolved. As you will see in this brief presentation, there is still room for discussing, and re-envisioning, with the use of murine models and data in humans, the concept and mechanisms of “rejection” of the foetal allograft.

But the question of the tolerance of the conceptions has evolved. First, we have known for a long time that we can and must dissociate local and systemic events, but this idea has not yet fully penetrated the thinking of the general public.

It is possible to observe rejection of paternal strain tissues after preimmunisation of the mother without compromising pregnancy4,5 (in fact, the babies born in such situations are in most circumstances heavier), and we have personally verified that.6

It ensues that systemic immune responses (which we do not want at all to negate, having taken a rather indisputable part in their discovery7) are, at best, a testimony of maternal recognition of the foeto-placental unit.

Indeed, pregnancy is perfectly possible in the absence of humoral (antibody) response.8,9 Indeed, if tolerance to the foetus was alloantibody mediated, it would be an exception, because tolerance is MHC restricted, and the Nobel prize awarded to Zinkernagel and Doherty recognizes the consequences of that phenomenon. With rare exceptions, antibodies are not MHC restricted, and attempts to say that tolerance was antibody mediated should be in immunology history. This is not completely true, since there is not yet a satisfactory explanation of the enhancement phenomenon, possibly because of the controversy over IgG1/IgG2 in mice, which uselessly shifted the debate-enhancing antibodies in rats, are part cytotoxic.

Similarly, we do not negate, of course, the existence of systemic suppressor cells,7 but pregnancy is perfectly viable in animals experimentally depleted of these.10

The repeated enunciation in France that pregnancy depends on an alloantibody and suppressor
T-cell-mediated immune response is simply outdated since around 1979–1981, and claims that unfortunately are expressed in mainstream immunology meetings and in print that “reproductive immunology has to teach the mainstream immunologists how the immune system works” as a “facilitation reaction” with “important role for enhancing antibodies... and suppresser cells” have disastrous effects:

- First, mainstream immunologists repeatedly dismiss, in a more and more irritated fashion, such prepostorous advances, and, as such, would consider as a backlash the whole field as not serious;
- Second, those claims have led some clinicians to exacerbate the role of maternal antibodies, clouding unconsciously the issue of early pregnancy loss.
- Third, they distract from the main advances that have indeed been going on, leading to molecular understanding of the field.

For indeed, there has been progress, such as Tom Wegmann’s seminal idea that possibly we should re-envisage our view of the immune system in pregnancy: instead of seeing the foetus as confronted by a threat of rejection by its mother—the “immunotrophic” concept postulated from a discovery made with the murine abortion model (the CBA × DBA/2 system),11,12 we recognize that immune reaction could be in some cases beneficial for fetal survival.13

Then, it ensued the demonstration in vitro, then in vivo, that indeed T-cell derived cytokines were growth factors for the placental trophoblasts,14 followed by in vivo demonstration of T-cell control of placental growth15,16 and treatment of a murine abortion model (CBA × DBA/2) by purified or recombinant cytokines.17

In the very same period, David Clark had made the discovery that this precise model (CBA × DBA/2) was deficient in local, decidual associated suppressor factor,18 and later on showed that this factor was molecularly related to TGF-b2.19

We are now faced with events occurring mostly locally, and with the concept of a cytokine network at the foeto-placental interface. The events are in fact different whether one looks at parturition (where there is good evidence for involvement of interleukin [IL]-1, IL-6, IL-8, and tumor necrosis factor [TNF]20–23,25) or the established pregnancy which is indeed immunologically characterised by transient acceptance of relatively weakly immunogenic tumor grafts of parental strain origin in the first pregnancy, linked to a T-cell anergy state and suppresser T-cell mediated multi-pregnancy induced tolerance to paternal alloantigens.26–29

In the latter case, the close examination by polymerase chain reaction (PCR) of cytokine profiles shows that it is more complicated than initially thought, with variations throughout pregnancy of which the physiological significance is yet unclear,30 and which we will not detail here in the human. Similarly, the profiles are not simple in mice.131

And we now come to the topic of this communication, early pregnancy. Nowhere is the understanding of the cytokine network more interesting, and nowhere has it led to more surprising discoveries, opposed to the paradigm of the acceptance of the foetal allograft, and so far away from the initial framework (which was indeed once useful) of the facilitation reaction.

Early implantation requires “inflammatory” molecules, the consequence, in part, of a quasi-inflammatory reaction locally, followed by an immediate return to non-inflammatory conditions. It is probable, from what we know in animal models, that each of these steps are absolutely required for further successful pregnancy. But before that, a word is required about the antigenicity of the embryo in mice and humans.

AN ALLOGRAFT?

It is a tautology, a “Lapalissade” to say that to be alloantigenic, an embryo must express foreign tissues antigens. This is not totally true. Failure to express major histocompatibility antigens has another counterpart. It activates cells which are what Charlie Loke calls the most primitive immune system,31 the Natural Killer cells (NKs). That it was so is a relatively recent discovery, which owes a lot to the theory of the “missing self” originally proposed by Klas Karre.32 Therefore, it ensues that the trophoblasts cannot theoretically be completely neutral, otherwise there would be an NK-mediated rejection reaction: the NKs express two sorts of receptors, which are not yet fully understood, albeit an impressive body of evidence is accumulating (see Immunological Reviews 158). Killer Activating Receptors (KARs), whose engagement, in a
neoclassical fashion, triggers NK lytic pathways (and possibly others, such as cytokine secretion, especially when the Killer Inhibitory Receptors [KIRs] are engaged). The second, and most important, are KIRs. The KIRs are activated when engaged by the reconnaissance of the presence of MHC molecules (most often in a given minor histocompatibility antigen context), which explains "easily" some "oddities" which we published for the sake of honesty on the CBA xDBA/2 system and which were then rejected by proponents of the MHC-T-cell duo theory (proponents for whom we, incidentally, have the greatest respect), triggered so as to inhibit NK lytic pathway.

An added level of complexity for immunologists and clinicians is that while MHC is (relatively) conserved through evolution as a member of the super Ig family, KIRs and KARs are not at all related between mice and humans. Why?

Somehow, the trophoblast faces a dilemma: express MHC, which might trigger a classical T-cell-derived rejection reaction, or repress completely MHC, which will defuse the KIRs and trigger NK-mediated rejection.

This dilemma is partly solved in the human. The trophoblast expresses, as far as its extravillous component is concerned, a specific antigen, HLA-G, with no variations (or a few amino acids) from one individual to the other, but it is now known that it has some variability. HLA-G has little peptide presenting capacity (but it does have it!), and, as a truncated molecule, it is not recognised as "polymorphic." (It has few antigenic disparities between individuals, if any, that can be "seen" by T-cell receptors, whereas it certainly inhibits NK function. Recent experiments by Strominger, Lopez Bottet et al, Ramensee, and Le Bouteiller confirm the earlier ones of Kovatts and Loke.)

The situation would thus seem simple in humans: the extravillous trophoblast is where interaction with decidual lymphocytes takes place and therefore where NK and NK-like cells accumulate and are defused. The lack of classical MHC molecules would render the trophoblast a non-target and a non-inducer of a classical T-cell-mediated response. In addition, there is no bioactive IL-2 in decidua, albeit an IL-2-like material has been traced.

The situation is more complex than that, even for those who would like to make an exception in the case of primates: in humans, the expression of HLA-G is restricted to extravillous cytotrophoblasts, villous cyto-, and syncytiotrophoblasts that are uniformly MHC-negative, but a mirror situation is observed in baboons, and in the rhesus monkey, HLA-G homologue is a pseudo gene (although its function could be exerted by a yet uncharacterised protein). So, this is already a snag.

Two further snags are to be discussed: First, how could the totally MHC-negative human syncytiotrophoblast, a fact not dismissed since its description by Faulk, not trigger NK-mediated lysis? This is as yet unexplained by proponents of the HLA-G paradigm. Second, we now know that HLA-C is expressed by extravillous trophoblast. It has a restricted polymorphism and interacts with NKs, but the theory must somehow be twisted to make it act as purported for the monomorphic HLA-G, for despite a certain paucity of reports, there are T-cell-mediated responses to HLA-C.

Finally, in all species other than the primates, there is expression of classical, polymorphic class I molecules, generally precisely on the layers of the placenta that confront the maternal immune system (44,45,46). In mice K, D, and L are expressed on the spongiotrophoblast, perfectly accessible to antibodies and cells. The same is true in horses for equine leukocyte antigens, in pigs for swine leukocyte antigens, and, according to Twink Allen, in elephants.

There are three alternative hypotheses for the problem:

- One is that man is man, or, as Peter Johnson wrote (The Immunologist, 1996:4;p172), "differences in placentation between humans, rodents, and other species can make direct comparisons largely meaningless."
- The second one is, as Leslie Brent says when discovering the status of HLA-G in other species, that HLA-G suppresses as would any proper MHC-ligand in the proper cells (that is, by the sort of experiments in which the missing self was discovered to be true) and that HLA-G has likely another function, since there is no equivalent in mice, etc, that warrants its evolutionary appearance (a specific peptide or hormone intracellular carrier, for example, yet uncovered).
The third one is that there are in other species functional equivalents of HLA-G, which could reside in the already discovered monomorphic or poorly polymorphic MHC class I molecules.

None of these theories, incidentally, is mutually exclusive.

However, for class II (I-α in mice, HLA-DR in humans), the situation is more simple. In no species do the trophoblast layers express class II alloantigens, and expression of class II on trophoblasts induced by azacytidine results in regular abortion in mice.\(^{47}\)

Why? The question is unsettled, since the mechanisms have not been explored, but one should recall here in the same vein that expression of class I in mice is restricted to spongiotrophoblast, with no expression being found on labyrinth. Indeed, derepression of class I expression in the labyrinth is found in interferon γ induced abortion, but one does not know if it is a consequence or a cause of abortion.\(^{48}\) So, we are in quicksand about the “allograft status of the embryo.”

As far as the preimplantation embryo is concerned, it is simpler to say that there is no class I expression (nor class II) whatsoever on the gametes, blastocysts, extracellular cell mass or ectoplacental cell cone (EPC) in any species, including, when studies could be done, humans.\(^{49}\) This is somehow troublesome for some aspects of the recent HLA-G theories: the negative EPC cells certainly are facing a very important NK accumulation.\(^{50}\) And yet, the EPC resists perfectly well NK-mediated lysis. Conversely, aborted embryos in many species at that early stage display MHC expression on EPC cells.

The solution may be simple: at that stage, the cells of the EPC are intrinsically resistant to cell-mediated lysis,\(^{51-53}\) most probably because cell-mediated death involves target cell participation and the pathways of apoptosis. Like other cells secreting high amounts of steroids, trophoblasts are resistant to steroid-induced apoptosis and thus to cell-mediated death. However, they are sensitive to LAKCs mediated cell death,\(^{54}\) but for that we will see that there is in addition a lot of immunosuppressive material in the vicinity, and that abortion might not be mediated by trophoblast death. Such an immunosuppression takes place after implantation, which on the contrary calls for inflammation, and is a Th1 response.

### Inflammation and Implantation

In France, the early stages of pregnancy are difficult to study in humans, despite advances in medically assisted reproduction. The Loi Huriet requests full informed consent before taking a biopsy sample, and in the very “sensitive” area of reproductive tract organs, this is quite a deterrent, as opposed to the ease with which blood samples are obtained.

In the implantation period, where some events are very transiently associated with blastocyst adhesion and initial invasion of uterine walls, it is, of course, inconceivable and unlawful to deliberately transfer an in vitro fertilisation (IVF) for the sole purpose of aspiration, or, even worse, (total) removal of uterine tissues for immunological investigations. As stated, animal models are not necessarily fully pertinent, and thus Y.W. Loke said at the 15th World Congress on Fertility and Sterility in Montpellier that the only valid model for human pregnancy is “the human species itself.”

But, this does not solve fully the problem of the periimplantation period. Macaques are not fully relevant, because of antigenic disparities, like baborons, at the trophoblast level with human situation, and chimpanzees are an endangered and protected species of extremely limited availability. For these reasons, in vitro alternative models have been developed, which may or may not be totally relevant, complemented by studies in animals, mostly in mice.

### Animal Studies

The studies performed in animals have revealed a salient and totally unexpected aspect contrasting with the established pregnancy: the preimplantation uterus undergoes an “inflammatory-like” reaction, with transient influx, after mating in mice and rats, there of lymphocytes and macrophages, and seminal fluid is required, since this is not seen in females with fallopian tubal ligation or in vasectomised males.\(^{55-57}\) As expected, an increased secretion of IL-1α, IL-1β, TNF α, IL-6, and mRNA is detected in the uterus, paralleled by the pregnancy-associated continuous rise in production of CSF-1 which starts by then.\(^{58,59}\) Except for CSF-1, those inflammatory cytokines return to basal levels
by days 3 and 4, but CSF-1 secretion continues to increase throughout pregnancy. In rats, local injection of the PAF-acether antagonist BN 50081 completely prevents implantation, whereas the untreated contralateral horn is fully receptive.

Three cytokines are, in our opinion, crucial, as established in mutant or "knock-out" animals: CSFs, IL-1, and LIF.

**CSFs (CSF-1, S-CSF, GM-CSF)**

CSF-1 deficient oSP (osteopetrotic, or op/op) mutant mice have a profound pregnancy defect, and males have also reproductive functions. CSF-1 receptor, c-fms, is expressed after the 2-4 cell stage until the EPC outgrowth and later the spongiotrophoblast and is also found at very high levels in the maternal then the primary decidual zone and finally in the sole decidua basalis. S-CSF and its receptor (c-kit, which maps in the dominant white spotting W locus in the mouse), is also important, since mutations in the W locus result in sterility in mice, often associated with abnormal embryonic development.

GM-CSF was the first cytokine with IL-3 involved in the immunotrophic theory and is found in the preimplantation uterus. Tartakowsky has shown that culture of CBA x DBA/2 embryos (a murine abortion model) in GM-CSF- or CSF-1-containing medium partly corrects the deficient implantation rate seen when transferring these embryos in CBA/J mothers. GM-CSF receptor is expressed in the EPC and later spongiotrophoblast cells and in the early implantation uterus. Recently, in an elegant series of experiments, Robertson implied the inflammatory response and more particularly the GM-CSF. In agreement with studies conducted to prove the immunotrophic theory, she found that GM-CSF-deficient mice have smaller litter sizes and placental weights than controls and implies GM-CSF and components in the semen ejaculate in hyporesponsiveness to paternal alloantigens.

**IL-1**

IL-1 might play a mandatory role in pregnancy. According to Simon and Polan, IL-1 receptor antagonist completely prevents successful implantation in mice. However, Colin Stewart et al. (Stewart, personal communication and answers at many meetings) have examined at the Roche Institute for Molecular Biology IL-1 deficient mice with perfectly normal reproductive function, nor did we obtain implantation failure in mice by in vivo injection of neutralising anti-IL-1 antiserum.

**LIF**

By gene knock-out technology, Stewart has elegantly shown that maternal production of LIF is mandatory for successful implantation: LIF-deficient mice, obtained by "gene knockout" are fertile, but sterile. LIF+/LIF- embryos implant in normal CD1 recipient mice, but LIF +/-LIF- embryos do not implant in LIF-/LIF- mice. The defect is corrected by recombinant HILDA LIF via an osmotic pump.

**Human Studies**

**IL-1**

IL-1 α, β expression is found in human endometrial epithelium throughout the menstrual cycle, reaching a peak in the late luteal phase. Immunohistochemistry shows that IL-1 receptor antagonist in human endometrial cells is at higher levels during the follicular phase than during the early and mid luteal phases. IL-1 is also found in trophoblasts and IL-1 receptor type 1 on syncytiotrophoblasts, suggesting an autocrine and paracrine role of IL-1 in human implantation. Indeed, it has been reported that IL-1 is a modulator of the decidualisation itself, and it is also controlling hCG production by human trophoblasts in culture. The production and role of IL-1 by early human embryos is controversial; some authors made it a predictor of successful implantation in humans, while others did not detect it at all. Differences in culture media, conditions, etc. obviously could be invoked. In a co-culture of endometrial epithelial cells and or stromal cells with embryos, IL-1, IL-1 receptor antagonist, and IL-1 receptor type 1 were found in about 56% of cells from embryos cultured with epithelial cells but not stromal cells, apparently correlated with pregnancy success.

**LIF**

LIF is present in human endometrium, with consensus for its expression in the second part of the cycle, and during pregnancy, this in variance with mice. Optimal uterine secretion coincides with the implantation window and expression of...
TABLE 1. Endometrial HILDA/LIF production index in fertile women

| HILDA/LIF production index | Cycle day | Age (years) | Number of successful pregnancies | Last pregnancy (years) |
|---------------------------|-----------|-------------|----------------------------------|------------------------|
| 1.05                      | 20        | 50          | 2                                | 18                     |
| 1.66                      | 6         | 36          | 2                                | 4                      |
| 1.94                      | 14        | 41          | 2                                | 20                     |
| 2.21                      | 10        | 37          | 4                                | 5 weeks                |
| 2.22                      | 20        | 41          | 2                                | 1                      |
| 2.28                      | 7         | 32          | 2                                | 9                      |
| 2.35                      | 22        | 34          | 2                                | 10                     |
| 3.16                      | 10        | 36          | 1                                | 12                     |
| 3.27                      | 18        | 32          | 1                                | 1                      |
| 3.39                      | 10        | 41          | 5                                | 14                     |
| 3.85                      | 19        | 35          | 2a                               | 6 weeks                |
| 4.06                      | 19        | 36          | 2                                | 12                     |
| 4.51                      | 22        | 32          | 2                                | 4                      |
| 4.57                      | 37        | 30          | 1                                | 5                      |
| 6.48                      | 11        | 38          | 2                                | 8                      |
| 9.95                      | 24        | 41          | 1b                               | 1                      |

*Median = 3.22.

*Plus one pregnancy loss before successful pregnancy.

From: Delage G, Moreau J-F, Taupin J-L, et al: In vitro endometrial secretion of human interleukin for DA cells/leukaemia inhibitory factor by explant cultures from fertile and infertile women. Hum Reprod 10:2483–2488, 1995.

We have found women with quantitative defects in LIF production; we have observed such a deficiency in women with successful IVF but confirmed sterility. We have now confirmed those data, obtained in a culture system, by both immunohistochemistry, using polyclonal antibodies, and evaluation of LIF production by isolated uterine epithelial cells.

Tables 1, 2a, 2b, and 3 show typical LIF production indexes in fertile and infertile women, as defined, in an in vitro assay system, by the index of LIF production by explant cultures in culture supernatants from Day 5 and Day 1. It is interesting to note that in women whose most recent reproductive event was a pregnancy loss, there are a great number with a low LIF production index (Table 3), the indexes being very low for women with recurrent implantation failure or recurrent unexplained sterility (Tables 4 and 2b). The existence of a low producer of LIF has been confirmed ex vivo by uterine flushing and ELISA assays.

As stated above, we wanted to have a totally objective measure of LIF production in situ by the human endometrium. We therefore set up the technique of selective separation digestion of glandular and stromal endometrium, after a Cornier of Frydman pipette biopsy sample, and using polyclonal antibodies raised in cooperation with J Mar-
TABLE 2A. Endometrial HILDA/LIF production index in infertile women with recurrent implantation failure

| HILDA/LIF production index | Cycle day | Age (years) | Number of IVF with embryos | Number of pregnancies | Last pregnancy (years) |
|---------------------------|-----------|-------------|-----------------------------|-----------------------|-----------------------|
| 0.96                      | 10        | 34          | 3                           | 1 (EUP)               | 15                    |
| 0.97                      | 5         | 29          | 20                          | 3 (2 PL, 1 EUP)       | 5                     |
| 0.97                      | 21        | 34          | 8                           | 2 (1 EPT, 1 EUP)      | 4                     |
| 1.01                      | 6         | 34          | 2*                          | 1 (PL)                | 9                     |
| 1.22                      | 15        | 35          | 3                           |                       |                       |
| 1.41                      | 10        | 39          | 1*                          |                       |                       |
| 1.49                      | 18        | 33          | 2                           |                       |                       |
| 1.68                      | 9         | 36          | 6                           |                       |                       |
| 1.69                      | 21        | 36          | 2                           |                       |                       |
| 1.70                      | 7         | 34          | 4                           |                       |                       |
| 1.74                      | 14        | 33          | 4                           | 1 (EUP)               | 10                    |
| 2.45                      | 18        | 38          | 6                           |                       |                       |

*Median = 1.45.

**Plus GIFT.

EUP = extrauterine pregnancy; PL = pregnancy loss; EPT = elective pregnancy termination.

From: Delage G, Moreau J-F, Taupin J-L, et al.: In vitro endometrial secretion of human interleukin for DA cells/leukaemia inhibitory factor by explant cultures from fertile and infertile women. Hum Reprod 10:2483–2488, 1995.

TABLE 2B. Endometrial HILDA/LIF production index in infertile women with unexplained primary sterility

| HILDA/LIF production index | Cycle day | Age (years) |
|---------------------------|-----------|-------------|
| 1.22                      | 12        | 41          |
| 1.28                      | 6         | 30          |
| 1.39                      | 8         | 30          |
| 1.48                      | 6         | 33          |
| 2.70                      | 13        | 34          |

*Median = 1.39.

From: Delage G, Moreau J-F, Taupin J-L, et al.: In vitro endometrial secretion of human interleukin for DA cells/leukaemia inhibitory factor by explant cultures from fertile and infertile women. Hum Reprod 10:2483–2488, 1995.

believe we have an objective assay that can complement immunohistochemistry, analysis on cytology smears, and in vitro production in explant culture.

It is important, in this context, to emphasize that recombinant human HILDA LIF is available, and women could benefit from substitutive therapy. While we feel confident enough to go forward with such a procedure, the clinicians would prefer we first correct a defect outside pregnancy before going further on.

This leads to questions about the mechanisms of action of LIF and, indeed, the inflammatory response. It is our working hypothesis that the local inflammation is required for optimal expression on placental trophoblast and uterine decidual cells of adhesion molecules, which are necessary for implantation to occur, such as trophoblast laminin and fibronectin receptors, and specifically the $\alpha 1 \beta 1$, $\alpha 5 \beta 1$, $\alpha 6 \beta 1$, and $\alpha 6 \beta 4$ integrin heterodimers, and expression of their receptor molecules (laminin and fibronectin) in the perimplantation as well as first trimester uterus with specific trophoblast down-regulation of expression of the $\beta 4$ integrin and up-regulation of the $\beta 1$, $\alpha 5$, $\alpha 1$ subunits during the invasion process.91-93

We believe that an important part of early pregnancy loss is due to abnormal processes of this early reaction. It is noticeable that dysregulations are already known, such as in preeclampsia, where trophoblasts do not down-regulate $\beta 4$, nor is $\alpha 1$ up-regulated.94 In addition, the effects of cytokines on the matrix degrading protease are also to be encompassed in that respect.31

TNF

There is early and temporary expression of relatively high levels of TNF by invading extravillous trophoblasts95 which could, as seen in other systems, up-regulate adhesion molecules and selectin.96 This inflammatory reaction, however, has to be then very quickly down-regulated, since during the early post implantation phase it is abortogenic. There are many other cytokines that we will not discuss here, for sake of space, but we cannot not mention here CSF-1 and its receptor, whose programme is very parallel to what is seen in rodents and which has a similar role.97-101 mainly as placental trophoblast growth factor acts as the other immunotrophic cytokine, IL-3102 In this commu-
IMMUNOPATHOLOGY OF EARLY PREGNANCY

TABLE 3. Endometrial HILDA/LIF production index in women whose last reproductive event was a pregnancy loss

| HILDA/LIF production index* | Cycle day | Age (years) | Number of successful pregnancies | Last successful pregnancy (years) | Number of pregnancy losses | Last pregnancy loss (years) |
|-----------------------------|-----------|-------------|---------------------------------|----------------------------------|---------------------------|--------------------------|
| 0.98                        | 15        | 38          | 0                               | 0                                | 1                         | 12                       |
| 1.12                        | 15        | 31          | 0                               | 0                                | 1                         | 4                        |
| 1.20                        | 19        | 34          | 2                               | 11                               | 2                         | 3 and 2                  |
| 1.79                        | 9         | 35          | 0                               | 0                                | 2                         | 2                        |
| 1.86                        | 6         | 33          | 0                               | 0                                | 1                         | 16                       |
| 1.89                        | 20        | 34          | 0                               | 0                                | 4                         | 1                        |
| 1.98                        | 13        | 36          | 3                               | 3                                | 1                         | 1                        |
| 2.33                        | 19        | 38          | 2                               | 6                                | 1                         | 4                        |
| 2.38                        | 19        | 41          | 2                               | 13                               | 1                         | 1                        |
| 2.43                        | 22        | 32          | 0                               | 0                                | 2                         | 3                        |
| 2.45                        | 12        | 35          | 0                               | >20                               | 0.5                       |                         |
| 2.79                        | 14        | 41          | 1                               | 5                                | 1                         | 3                        |

*Median = 1.94.
From: Delage G, Moreau J-F, Taupin J-L, et al.: In vitro endometrial secretion of human interleukin for DA cells/leukaemia inhibitory factor by explant cultures from fertile and infertile women. Hum Reprod 10:2483–2488, 1995.

TABLE 4A. Endometrial HILDA/LIF production index in infertile women with recurrent implantation failure

| HILDA/LIF production index* | Cycle day | Age (years) | Number of IVF with embryos | Number of pregnancies | Last pregnancy (years) |
|-----------------------------|-----------|-------------|-----------------------------|-----------------------|------------------------|
| 0.96                        | 10        | 34          | 3                           | 1 (EUP)               | 15                     |
| 0.97                        | 5         | 29          | 20                          | 3 (2 PL, 1 EUP)       | 5                      |
| 0.97                        | 21        | 34          | 8                           | 2 (1 EPT, 1 EUP)      | 4                      |
| 1.01                        | 6         | 34          | 2b                          | 1 (PL)                | 9                      |
| 1.22                        | 15        | 35          | 3                           | 0                     |                         |
| 1.41                        | 10        | 39          | 1b                          | 0                     |                         |
| 1.49                        | 18        | 33          | 2                           | 0                     |                         |
| 1.68                        | 9         | 36          | 6                           | 0                     |                         |
| 1.69                        | 21        | 36          | 2                           | 0                     |                         |
| 1.70                        | 7         | 34          | 7                           | 0                     |                         |
| 1.74                        | 14        | 33          | 4                           | 1 (EUP)               | 10                     |
| 2.45                        | 18        | 38          | 6                           | 0                     |                         |

*Median = 1.45.
*Plus GIFT.
EUP = extraterine pregnancy; PL = pregnancy loss; EPT = elective pregnancy termination.
From: Delage G, Moreau J-F, Taupin J-L, et al.: In vitro endometrial secretion of human interleukin for DA cells/leukaemia inhibitory factor by explant cultures from fertile and infertile women. Hum Reprod 10:2483–2488, 1995.


cication, however, we would like to mention tau and placental interferons.

Placental Interferons

Corpus luteum maintenance in ovine does not depend on non-chorionic gonadotropin, but on trophoblastin whose trophoblast secretion occurs in the periimplantation, also named ovine trophoblast protein (oTP). It has pleiotropic activities. It exerts a wide variety of effects, such as local antiviral properties, preparation of the uterus for optimal implantation by promoting 2 5 A synthetase activity, and local cytostatic properties, making it an ideal candidate for early immune suppression. It is likely to be involved in control of the Th1/Th2 balance. It is in fact an interferon of the new
TABLE 4B. Endometrial HILDA/LIF production index in infertile women with unexplained primary sterility

| HILDA/LIF production index* | Cycle day | Age (years) |
|-----------------------------|-----------|-------------|
| 1.22                        | 12        | 41          |
| 1.28                        | 6         | 30          |
| 1.39                        | 8         | 30          |
| 1.48                        | 6         | 33          |
| 2.70                        | 13        | 34          |

Median = 1.39.

*From: Delage G, Moreau J-F, Taupin J-L et al.: In vitro endometrial secretion of human interleukin for DA cells/leukaemia inhibitory factor by explant cultures from fertile and infertile women. Hum Reprod 10:2483-2488, 1995.

tau interferon family. When such trophoblasts exist, they are constitutively secreted at very high doses locally by perimplantation trophoblast only during the perimplantation phase. It is non abortogenic (of course, it maintains pregnancy) and non toxic/cytostatic for trophoblast, which secretes it, while ? interferon at high doses is an abortifacient in a variety of animal models. The quest is still ongoing for human equivalents, but not conclusively, despite suggestive evidence109, so one speculates that its functions could in fact be exerted by omega interferons.

**Th1/Th2 BALANCE AND LOCAL IMMUNOSUPPRESSION**

As soon as one completes the implantation period, in humans and in mice, an abnormal Th1 reaction to trophoblast causes early pregnancy loss, recurrent spontaneous abortion, and infertility. Several mechanisms are involved. For example, the HELLP syndrome (hemolysis, elevated liver function tests, and low platelet counts) is linked to an elevated IL-12 serum level and possibly an enhanced IL-2 level in the serum110,111 of unclear origin, since despite recent molecular evidence for the contrary112, no IL-2 is assumed to reside in decidua and placenta. This is especially important, since in a variety of animal systems, activated NKs (by IL-2 or IL-4, e.g., LAKCS), are abortifacient, and indeed LAKCS can lyse trophoblast cells in vitro, but not normal NKs or CTls. Thus, there has been a quest as indeed “built-in” in the “allograft model” for local immunosuppression for the mechanisms of early pregnancy loss and its control.

The Th1 cytokines, be they IL-2, interferon γ, or TNF, are abortifacient in vivo, the latter at any stage of pregnancy, in abortion-prone and non-abortion-prone systems117,113,114,116,117 Interferon γ and TNF act in synergy. Their control is exerted by a variety of non-cytokinic mediators (prostaglandins, 1-25 OH dihydroxy cholecalciferol in the decidua, placental low MW suppressor factors), as well as by suppressive cytokines such as TJ6 (the “pregnancy-associated cytokine”) which has been cloned initially in mice. Monoclonals against TJ6 are abortifacient at early stages, and deficient expression of TJ6 is reported to be associated with pregnancy loss in human, especially if the defect is on CD 19- and CD 56-positive peripheral and decidual lymphocytes. Such a defect could be predictive of risk of pregnancy loss.118-121 Another regulator which is, like TJ6, progesterone dependent is the progesterone induced blocking factor (PIBF), secreted by activated T lymphocytes.122 PIBF alters the Th1/Th2 balance towards Th2 and corrects activators of NKK (poly IC) and natural (CBA × DBA/2) induced abortion.123-125 Its expression by maternal lymphocytes has been shown to correlate with (perhaps even require) implantation.126 The decidua-associated transforming growth factor β-2 analogue9 is secreted by uterine non-T, non-B cells in mice and decidual transforming growth factor β-2 secreting cells in humans.126 It is a potent immunosuppressive factor, and it has been reported that its production was defective in women with repeated miscarriages.126 Finally IL-10 is clearly important, since in animal models pregnancy is very obviously a Th2 phenomenon.127 In humans, it is secreted by placental trophoblasts and choriocarcinoma,128,129 and, interestingly, its secretion is enhanced by GM-CSF. IL-10 is also produced by isolated decidual cells.130 In mice, its secretion is more controversial.131,132 But, in murine models, IL-10 with IL-3 suppress local action of IL-2 and gamma interferon, biasing the maternal immune response towards a Th2 profile. Deneyes (UCL, Bruxelles, Belgium) has shown that in pregnant women, TNF was down-regulated and IL-4 up-regulated when measured in the serum, a pattern not seen in recurrent aborters (Deneyes, personal communication). Further proof of the role of IL-10 is the effect observed in infections. Leish-
mania biases the responses towards Th1, and there is less IL-10 but more pregnancy failure.133,134

**EFFECTOR MECHANISMS**

What are the exact mechanisms of early pregnancy loss? It is clear that interferon and gamma interferon are involved, very often as a consequence of either abnormal maternal recognition of the conceptus/semen, or local, undetected "a trés bas bruit" infection, with peculiar insistence on mycoplasma.17,108,133–136 The data of Hill strongly suggest it is also the case in humans, e.g., Th1 immunity causes recurrent abortions and sterility, with emphasis on gamma interferon.137,138 But what are the exact mechanisms?

First, the cells and the "immunological events" need to be reconsidered, even for immunotrophism; interest is leaning now toward NKs rather than T cells. Anne Croy has shown that placentae of NK-deficient mice are grossly hypotrophic, leading to premature foetal death,139 shifting the "immunotrophic" paradigm from T cells to NK cells. Thus, the production of immunotrophic cytokines might be in fact mostly (but not exclusively) under direct control of NK cells more than T cells, and "allorecognition" of pregnancy might, in fact, be exerted by these NKs rather than in a classical fashion by T cells. In consequence, a hitherto little suspected role could be envisaged for HLA-G: the control of the production of immunotrophic cytokines and not solely the local defusing of NK mediated cytolytic functions (a role that was forecasted by Y W Loke31).

In the CBA x DBA/2 model of natural immune abortion, together with the poly IC 12 U model, (an NK activator),142 we explored the role of NKs in cooperation with P. Kourilsky.140,143,144 Anti MHC H-2d (BALB/c) alloimmunisation prevents the effect of Poly IC or Poly I Poly C12 U as well as CBA x DBA/2 fetal loss. But, not all selective alloimmunisation prevents the CBA x DBA/2 foetal loss, and the genetic patterns of the effective immunising splenocytes (MHC restricted, minor loci dependent), albeit apparently representing a single trait,12,141 are so odd to comprehend in terms of "classical" T-cell recognition (BALB/c works in CBA xDBA/2, B10,D2 does not–both are H-2d) that some T-cell and MHC proponents preferred to dismiss the data as non reproducible (which they were not, being reproduced at present by more than 20 labs, with significant developments) or (we quote) "absolute bullshit."

But, we then demonstrated that immunisation with Kd or Ld transfected L cells prevented resorptions and used that system to study the effect of amino acid mutations on the border of the pocket of the MHC molecule in that system. These were shown to affect recognition by maternal cells promoting optimal foetal survival when immunisation was performed using H-2d mutant transfected L cells. We then used various mutations that were artificially induced and localized on the border of the molecule’s groove or in the peptide binding site. Some H-2d mutations on the helix are recognized as foreign to H-2d and afford foetal protection against Poly IC. With P. Kourilsky, we wondered whether NK recognition of discrete determinants on the MHC molecule was not involved and, with help of Sylvie Delassus, Jean Pierre Abastado, Claude Roth, and Jos Even, we immunised CBA/J with L cells transfected with H-2d molecules mutant on positions 65, 69, which do not affect peptide presentation and 114 as a control. These protected against CBA x DBA/2 fetal loss, and, more important, enhanced IL-10 production in the supernatant of placental and decidual explants as already described using a commercial ELISA of high sensitivity, while treatment with asialo GM1 abolishes this effect.

At this stage, we propose the following working hypothesis: the NK cell repertoire is heteroclitic and consists of the recognition of selected altered domains of MHC molecules, whether that alteration results from mutations or allosteric conformational changes induced by (in the context of) background genes. Such a recognition either switches lytic/cytostatic pathway (macrophage activation, NK activation, TNF secretion, and balance of the CD4 system towards a Th1 profile) to IL-4 and IL-12 secretion by the NKs or pushes the system towards IL-10 secretion, and secretion by NKs or under the influence of NKs of the so-called "immunotrophic" cytokines. Thus, the concept would integrate both immunotrophism and the Th1/Th2 balance.

As far as the effector mechanisms are involved, it is already well known that TNF and interferon are causative and act in synergy,108,145,146 but other mediators have been implied, such as nitric oxide released by activated macrophages.147–150 There is
TABLE 5. Role of asialo GM1 + NK cells and macrophages in abortions

| Expt | Day 6.5 treatment | Day 7.5 treatment | Day 13.5 assay | N* resorptions/total | % abortions |
|------|------------------|------------------|----------------|---------------------|-------------|
| 1    | PBS              | PBS              | 8              | 23/56               | 41%         |
|      | PBS              | 1000 v TNF-α     | 8              | 43/60               | 72%         |
|      | PBS              | 2000 v TNF-α     | 8              | 57/64               | 99%         |
|      | anti-asialo GM1  | PBS              | 8              | 10/59               | 19%         |
|      | anti-asialo GM1  | 1000 v TNF-α     | 8              | 12/63               | 19%         |
|      | anti-asialo GM1  | 2000 v TNF-α     | 8              | 12/55               | 22%         |
|      | PBS              | PBS              | 16             | 43/101              | 43%         |
|      | PBS              | 1000 v γ-IFN     | 16             | 79/93               | 85%         |
|      | PBS              | 1000 v γ-IFN + TNF-α | 16 | 74/89 | 83% |
|      | anti-asialo GM1  | PBS              | 16             | 11/71               | 15%         |
|      | anti-asialo GM1  | 1000 v γ-IFN     | 16             | 12/98               | 12%         |
|      | anti-asialo GM1  | 1000 v γ-IFN + TNF-α | 16 | 89/104 | 86% |
|      | PBS              | PBS              | 16             | 36/88               | 41%         |
|      | PBS              | γ-IFN + TNF-α    | 8              | 65/80               | 81%         |
|      | PBS              | γ-IFN + TNF-α    | 8              | 14/55               | 25%         |
|      | SiO₂             | PBS              | 8              | 52/65               | 80%         |

*No represents number of pregnant mice per group.

**Significant increase in abortion rate, P < 0.005 by χ².

***Significant increase in abortion rate compared to PBS control, P < 0.005 by χ²; significant difference compared to lower dose of TNF-α, P < 0.05.

**Significant reduction in abortion rate by anti-asialo GM1 antibody compared to PBS control, P < 0.005 by χ².

*No significant boosting of abortion rate compared to PBS injected anti-asialo GM1-treated group.

1. Result from two independent experiments giving same result have been pooled.

2. γ-interferon (γ-IFN) significantly boosted abortion rate, P < 0.005 by χ².

3. TNF-α was given at 1000 v and 2000 v in separate experiments with γ-IFN and gave similar results; the data have been pooled for ease of presentation. The abortion rate was significantly boosted, P < 0.005 by χ².

4. Untreated CBA/J female mice mated to DBA/2 males.

5. 1000 v γ-IFN + 2000 v TNF-α significantly boosted abortion rate, P < 0.005 by χ².

6. CBA/J mice injected twice a week for 4 weeks with 100 mg/kg silicon dioxide before mating significantly reduced abortion rate, P < 0.05 by χ².

No doubt that asialo GM1+ cells or cells of the NK lineage are involved, since their transfer, once activated, causes abortion, and, since they do accumulate at the site of embryo resorption, their modulation positively (Poly IC activation) or negatively (anti-asialo GM1 treatment) influences parallel resorption rates.151–154 We have studied this problem with David Clark, taking into account the fact that TNF and gamma interferon are by themselves abortifacients, and performing cell deletion to learn the cellular mechanism triggered.155

In Table 5, intraperitoneal injection of TNF-α enhanced CBA x DBA/2 resorptions, but anti-asialo GM1 antibody both decreased the background rate and prevented the action of TNF-α. When we added γ-interferon, it had as expected an abortifacient effect, but that was not seen in NK-cell-depleted mice, suggesting that the Baines model149,150 γ-interferon (macrophages) activated to produce NO (embryo death was not correct). However, when γ interferon and TNF-α were administered together, more than 80% of the implanted embryos aborted. This confirmed the already observed synergy/codependence and suggested that in fact it might be crucial. Indeed, NK cell depletion suggested that TNF-α could not find enough NK-derived γ-interferon, while γ-interferon alone fails because macrophages which depend on NK-cell-derived γ-interferon have and thus did not produce TNF-α, and the intraperitoneally injected cytokine does not stimulate TNF-α production quickly enough.

The action of NKs or macrophage has been attributed to a direct killing of trophoblast. In silica treated macrophage-depleted mice, the abortion rate was reduced, but this treatment was ineffective in TNF-α plus γ-interferon treated animals, whose macrophage depletion was checked by the anti F4/80+ MoAb. Ovarian failure could have happened after silica, but this was ruled out since it should have caused abortions in 100% of cases, and the results were unaltered hormone replacement therapy treated animals.

The most logical target appeared to be the maternal uterine vascular endothelial cell, since the cytokines stimulate surface expression of procoagulant (fIIg/2-prothrombinase, which is distinct from tissue factor) and clotting. Table 6 shows that an-
### TABLE 6. Antibody to flg/2 prothrombinase prevents abortions in DBA/2-mated CBA/J mice

| Pretreatment group | Day 7.5 treatment | Day 13.5 assay | N resorptions/total | % abortion |
|--------------------|------------------|----------------|---------------------|------------|
| Control Rabbit IgG | nil 8            | 21/56a         | 38%                 |
| Control Rabbit IgG | γ-IFN + TNF-αb   | 8              | 48/55               | 87%        |
| Rabbit IgG anti-flg/2 | nil 9          | 3/66           | 4.5%               |
| Rabbit IgG anti-flg/2 | γ-IFN + TNF-αb  | 9              | 9/68                | 13%        |

*aResults from two independent experiments which gave the same result.

**1000 γ-IFN and 2000 u TNF-α was injected intraperitoneally.

Significant increase in abortion rate P < 0.001 compared to no cytokine control group, Fisher's Exact test.

Significant reduction in spontaneous abortion rate P < 0.001 compared to no cytokine control group, Fisher's Exact test.

Significant reduction in abortion rate P < 0.001 compared to cytokine-treated controls, Fisher's Exact test. No significant difference compared to anti-flg/2-treated mice which did not receive an injection of cytokines.

This model is of importance in discussing early human pregnancy loss. Of interest is the fact that a monoclonal antibody, BA11, prepared by R. Jalali in James Mowbray laboratory, blocks both classical and stress-mediated abortion.166,167

To finish, we would like to say we do not negate systemic events, we simply relativise them. As stated at the beginning of this paper, we do not negate the existence of allograft enhancement during pregnancy, nor peripheral T-cell hyporesponsiveness.26-29,67,168-170 We merely believe they reflect local events, for the most part, including the hitherto undiscovered placental suppressor factors. These, that we have suspected since 1980,171 have proved to be elusive.172-174 But with a continuous effort in mice,175-182 we have delineated the active moiety in human and murine placental supernatants, and this induces T-cell anergy181,182 in a very similar manner to staphylococcal enterotoxin,183 explaining, in our view, the T-cell unresponsiveness observed locally184 whose aforementioned phenomena (especially the report by Tafuri27) are in our opinion a mere reflection. In that respect, since the anergy is transient and reversible, it is interesting to note that optimal secretion of the factor in mice is seen by cells of the invasive ECP,172 and that there is a local deficiency in suppressor materials in the CBA x DBA/2 window of abortion.173

### CONCLUSIONS

There are several causes of early pregnancy loss, which we are only beginning to uncover. Several, as in the murine system, have different etiologies, but use a final "rejection-like" common pathway, where TNF and gamma interferon are important. But several others are likely to be due to NK failure.
TABLE 7. Effect of anti-granulocyte antibody on abortion rate

| Group | Day 6.5 treatment | Day 7.5 treatment | Day 13.5 assay | N resorptions/total | % abortion |
|-------|------------------|------------------|----------------|--------------------|-----------|
| 1     | Control rat IgG<sup>a</sup> | PBS | 8 | 17/57 | 30% |
| 2     | Rat anti-granulocyte<sup>b</sup> | PBS | 6 | 7/45 | 16% |
| 3     | Control rat IgG<sup>c</sup> | TNF-α + γ-IFN | 9 | 48/56 | 86% |
| 4     | Rat anti-granulocyte | TNF-α + γ-IFN | 7 | 8/57 | 14% |
| 5     | rIL-10 | rIL-10 | 5 | 2/42 | 5% |

<sup>a</sup>Monoclonal isotype control IgG 100 mg intraperitoneally as in Materials and Methods.

<sup>b</sup>Low endotoxin rat monoclonal IgG 100 mg intraperitoneally as in Materials and Methods.

<sup>c</sup>Reduction in abortion rate compared to group 1, *P = 0.072* by Fisher’s Exact test.

<sup>d</sup>TNF-α + γ-IFN given as in Tables 1 and 2. Significant increase in abortion rate compared to group 1, *P < 0.001* by χ² and Fisher’s Exact test.

<sup>e</sup>Significant reduction in abortion rate compared to group 3, *P < 0.001* by χ² and Fisher’s Exact test. Pooled result from groups 2 and 4, 15/102 = 15% abortion rate, significantly less than 30% rate in group 1, *P < 0.05* by χ².

<sup>f</sup>Significant reduction in abortion rate compared to group 1, *P < 0.001* by Fisher’s Exact test. No significant reduction compared to group 2 (*P = 0.096*) or pooled groups 2 and 4 (*P = 0.075*).

Clark DA, Chaouat G, Arck P, Mittruecker HW, Levy GA: Cytokine-dependent abortion in CBA X DBA/2 mice is mediated by the procoagulant fig/2 prothrombinase. In press.

Stoppacciaro A, Melani C, Parenza M, et al.: Regression of an established tumor genetically modified to release granulocyte colony-stimulating factor requires granulocyte-T cell cooperation and T cell-produced interferon γ. J Exp Med 178:151, 1997.

or misrecognition, hence improper cytokine secretion and lack of growth factors.

Several others are due to defects of the local inflammatory reaction (endometriosis, with preexisting high levels of TNF, is among these), and it is likely that, as in the LIF system, we will see discrete defects leading to definition of molecular abnormalities, with defects in adhesion molecules being involved.

Thus, early pregnancy loss and recurrent miscarriage, though due to “immunological-like” circuitry, are likely to be split up into different syndromes. It is our opinion in that respect that it is not at all surprising that only one woman out of 10 or 11 benefits from purely immunologic treatment. We have to be able to define which women will benefit by rigorous immunological criteria. (Unfortunately, the leukocyte immunisation saga has lead to many charlatan, unconscious, or preposterous thecisions, sometimes with disastrous consequences; women are not guinea pigs nor mice! We have always wondered what was the real basis for the MHC linked, disequilibrium antigen, as well as how a system like the CBA x DBA/2—which was described as minor loci dependent, MHC restricted and in which the good father (BALB/c) and the bad father (DBA/2) were both H-2d—could have been taken as an example of the proof for the need for absence of HLA homology! This is just an example, but we could—and, in fact, we are due to—write horrid things about anti-paternal antibodies, to illustrate what was said at this paper’s onset.)

This has to be said, because otherwise we learn from the discoveries of complex cytokine networks at present is that by unfounded treatments we are at risk of altering other useful pathways and induce the lack of remission of transient infertility.

The “stress” saga tells us about the importance of neuroimmunoendocrine pathways, which also should not be a surprise.

To the impatience of clinicians, we recall what H. Metzger said when he was cloning the IgE receptor: “Haste is waste.” We believe we have made significant progress in the past five years in understanding, with the help of animal models, in unraveling pathways which are operational indeed.

But, we now have to take into account that added level of complexity. We know it sounds difficult for clinicians, because we have a jargon, but we cannot escape the truth. Yes, a cytokine network is operating in early pregnancy, and, in certain cases, we might be found guilty if we were treating patients as if ignoring its existence.

REFERENCES

1. Medawar PB: Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol 7:320–338, 1953.

2. Brent L: A History of Transplantation Immunology. London: Academic Press, 1996.

3. Billingham RE: Immunobiological aspects of the foetal maternal relationship. In: Lachman P, Peters D (eds): Clinical Aspects of Immunology. 4th ed. Oxford: Blackwell Scientific, 1981.

4. Lannom JT, Dincostain JE, Fikrig S: Homograft immnunity in pregnancy. Lack of harm to fetus from sensitisation of the mother. Ann NY Acad Sci 99:706–718, 1962.
5. Mitchison NA: The effect on the offspring of maternal immunisation in mice. J Genet 5:406-411, 1953.

6. Monnot P, Chaouat G: Systemic active suppression is not necessary for successful allogenesis. Am J Reprod Immunol 6:5-8, 1984.

7. Chaouat G, Voisin GA, Escalier D, Robert P: Facilitation reaction (enhancing antibodies and suppressor cells) and rejection reaction (sensitised cells) from the mother to the paternal antigens of the conceptus. Clin Exp Immunol 35:13-24, 1979.

8. Rodger JC: Lack of requirement for a maternal humoral immune response to establish or maintain a successful allogeneic pregnancy. Transplantation 40:372-375, 1985.

9. Mattson R, Mattson A, Sulila P: Allogeneic pregnancy in B cell depleted CBA/Ca mice. Effects on fetal survival and maternal lymphoid organs. Dev Comp Immunol 9:709-717, 1985.

10. Mattson R, Holmdal R: Maintained allopregnancy in rats depleted of T cytotoxic/suppressor cells by OX8 monoclonal antibody treatment. J Reprod Immunol 1987.

11. Chaouat G, Kiger N, Wegmann TG: Vaccination against spontaneous abortion in mice. J Reprod Immunol 38:9-32, 1983.

12. Chaouat G, Kolb JP, Kiger N, Stanislawski M, Wegmann TG: Immunological concomitants of vaccination against abortion in mice. J Immunol 134:1594, 1985.

13. Wegmann TG: Fetal protection against abortions is it immunosuppression or immunostimulation? Ann Immunol Inst Pasteur 135D:309, 1984.

14. Athanassakis I, Bleackley RC, Paetkau V, Guilbert L, Barr J, Wegmann TG: The immunostimulatory effects of T cells and T cell lymphokines on murine foeto Derived placental cells. J Immunol 138:37-44, 1987.

15. Wegmann TG, Athanassakis I, Guilbert L, et al.: Maternal T cell reactivity as a positive determinant of placental growth and fetal survival. In: Belissario R, Mijeszewski G (eds.): Transplantation Disorders. Pre natal Detection, Treatment and Management. New York: Alan Liss, pp. 69-76, 1991.

16. Chaouat G, Menu E, Athanassakis I, Wegmann TG: Maternal T cells regulate placental size and fetal survival. Regional Immunol 1:143, 1988.

17. Chaouat G, Menu E, Dy M, Minkowski M, Clark DA, Wegmann TG: Control of fetal survival in CBA × DBA/2 mice by lymphokine therapy. J Fertil Steril 89:447-458, 1990.

18. Clark DA, Chaput A, Tutton B: Active suppression of host versus graft reaction in pregnant mice. VII. Spontaneous abortion of CBA × DBA/2 foetuses in the uterus of CBA/J mice correlates with deficient non-T suppressor cell activity. J Immunol 136:1668-171, 1986.

19. Clark DA, Flanders KC, Banwart D, et al.: Murine pregnancy decidua produces a unique immunosuppresive molecule related to transforming growth factor b-2. J Immunol 144-12:3008-3004, 1990.
37. King A, Loke YW, Chaouat G: NK cells and reproduction. Immunol Today. 18:64-66, 1997.
38. King A, Hiby SE, Verma S, Burrows I, Gardner L, Loke YW: Uterine NK cells and expression of trophoblast HLA Class I molecules. Am J Reprod Immunol 37:459-463, 1997.
39. Soubrain P, Zapitelli JP, Schaffer L: IL-2 like material is present in human placenta and amnion. J Reprod Immunol 12:225-234, 1987.
40. Stern PL, Bereford N, Friedman CI, Friedman CI, Stevens VC, Risk JM, Johnson PM: Class I like molecules are expressed by baboon placental syncytiotrophoblast. J Immunol 138:1088-1091, 1987.
41. Boyson JE, Iwanaga KK, Golos TG, Watkins DJ: Identification of rhesus monkey HLA-G ortholog. Mamu-G is a pseudo gene. J Immunol 157:5428-5437, 1996.
42. Faulk WP, Temple A: Distribution of beta 2 microglobulin and HLA in chorionic villi of human placenta. Nature (London) 262:799-802, 1976.
43. Faulk WP, McIntyre JA: Immunological studies of trophoblast markers, subsets and functions. Immunol Rev 75:139-175, 1983.
44. Allen WR, Kydd JH, Antczack DF: Successful application of immunotherapy to a model of pregnancy failure in equids. In: Clark DA, Croy BA (eds.): Reproductive Immunology. Elsevier pp. 253-261, 1986.
45. Singh B, Raghupathy R, Anderson DJ, Wegmann TG: The murine placenta as an immunological barrier between the mother and the fetus. In: Wegmann TG, Gill TJ (eds.): Immunology of Reproduction, 51st Banff conference. New York: Oxford University Press, pp. , 1983.
46. Wegmann TG, Mossman TR, Carlson G, Olingk O, Singh B: The ability of the murine placenta to absorb monoclonal anti fetal H-2 K antibody from the maternal circulation. J Immunol 122:270-277, 1979.
47. Athanassakis Vassiliadis I, Papanettheakis J: Modulation of class II antigens on fetal placental lads to fetal abortion. In: Chaouat G, Mowbray J (eds.): Biologie Cellulaire et Moléculaire de la Relation Materno Fétale. Paris: Editions INSERM John Libbey, pp. 69-81, 1991.
48. Mattsson R, Holmdahl R, Schenius A, Bernadotte F, Mattsson L: Placental MHC class I antigen expression is induced in mice following in vivo treatment with recombinant interferon gamma. J Reprod Immunol 19: 115-129, 1991.
49. Clark DA: Controversies in reproductive immunology. Crit Rev Immunol 11:215, 1991.
50. Croy BA, Gambel P, Rossant J, Wegmann TG: Characterisation of murine decidual Natural Killer cells and their relevance to the success of pregnancy. Cell Immunol 103: 315-326, 1985.
51. Croy BA, Rossant J: Mouse embryonic cells become susceptible to CTL mediated lysis after midgestation. Cell Immunol 104:355-365, 1987.
52. Zuckerman FA, Head JR: Murine trophoblast resist cell mediated lysis. I. Resistance to allospecific cytotoxic T lymphocytes. J Immunol 139:2856-2865, 1987.
53. Zuckerman FA, Head JR: Murine trophoblast resist cell mediated lysis. II. Resistance to Natural cell mediated cytotoxicity. Cell Immunol 116:274-286, 1988.
54. Drake BL, Head JR: Murine trophoblast cells are susceptible to Lymphokine Activated killer (LAK) cell lysis. Am J Reprod Immunol 16:114, 1988.
55. Noun A, Acker G, Chaouat G, Antoine JC, Garabedian M. Macrophages and T lymphocyte bearing antigens bearing cells in the uterus before and during ovum implantation in the rat. Clin Exp Immunol 78:434-438, 1989.
56. Kachkache M, Acker GM, Chaouat G, Noun A, Garabedian: Hormonal and local factors control the immune histochemical distribution of immunocytes in the rat uterus before conceptus implantation: Effects of ovariecotomy, fallopian tube section and RU 486 injection. Biol Reprod 45:860-865, 1991.
57. Mac Master MT, Newton RG, Sudhanski K, Dey, and Andrews G.K. Activation and distribution of inflammatory cells in the mouse uterus during the preimplantation period. J Immunol148. 1699-1705.1992
58. Sanford TR, De M, Wood G: Expression of colony stimulating factors and inflammatory cytokines in the uterus if CD1 mice during days 1 to days 3 of pregnancy. J Reprod Fertil 94:213-220, 1992.
59. Arceci B, Shanahan F, Stanley ER, Pollard JW: The temporal expression and localisation of colony stimulating factor (CSF-1) and its receptor in the female reproductive tract are consistent with CSF-1 regulated placental development. Proc Nat Acad Sci 86:8811-8818, 1989.
60. Pollard JW, Hunt JW, Wiktor Jedrzejezack W, Stanley ER: A pregnancy defect in the osteopetrotic (op/op) mouse demonstrates the requirement for CSF-1 in female fertility. Dev Biol 148:273-283, 1991.
61. Cohen PE, Chisholm O, Arceci RJ, Stanley ER, Pollard JW: Absence of CSF1 in osteopetrotic (csfmop/ csfmop) mice results in male fertility defects. Bio l Reprod 55:310- 317, 1996.
62. Pollard JW, Pampfer S, and Arceci RJ: Class III tyrosine kinase receptors at maternal fetal interface. In: Chaouat G, Mowbray J (eds): Biologie Cellulaire et Moléculaire de la Relation Materno Fétale. Paris: Editions INSERM John Libbey, pp. 81-91, 1991.
63. Tartakowsky B, Goldstein O, Ben Yair B: In vivo modulation of pre embryonic development by cytokines. In: Chaouat G, Mowbray J (eds): Biologie Cellulaire et Moléculaire de la Relation Materno Fétale. Paris: Editions INSERM John Libbey, pp. 81-91, 1991.
64. Robertson SA, Seamark RF: Uterine granulocyte macrophage colony stimulating factor in early pregnancy: cellular origin and potential regulators. In: Chaouat G, Mowbray J (eds): Biologie Cellulaire et Moléculaire de la Relation Materno Fétale. Paris: Editions INSERM John Libbey, pp. 113-121, 1991.
65. Robertson SA, Seamark RF, Guilbert LJ, Wegmann TG: The role of cytokines in gestation. Crit Rev Immunol 14:239-292, 1994.
66. Athanassakis I, Chaouat G, Wegmann TG: The effects of anti CD4 and anti CD8 antibody treatment on placental growth and function in allogenic and syngenic pregnancy. Cell Immunol 129: 1990.

67. Robertson SA, Mau VJ, Hudson SN, Tremellen KP: Cytokine leukocyte networks and the establishment of pregnancy. Am J Reprod Immunol 37:438–443, 1997.

68. Simon C, Frances A, Piquette GN, et al.: IL-1 receptor antagonist prevents successful implantation in mice. Endocrinol 134:521–528, 1994.

69. Stewart C, Kaspar P, Brunet LJ, et al.: Blastocyst implantation depends on maternal expression of Leukemia Inhibitory Factor. Nature 359:76–79, 1992.

70. Simon C, Piquette GN, Frances A, Polan ML: Localisation of Interleukin 1 type 12 receptor and interleukin 1beta in human endometrium throughout the menstrual cycle. J Clin Endocrinol Metab 77:594–595, 1993.

71. Kauma S, Matt D, Strom S, Eirman D, Turner T: Interleukinbeta (IL-1beta), human leucocyte antigen HLA-DR alpha, and transforming growth factor beta (TGF beta) expression in endometrium, placenta and placental membranes. Am J Obstet Gynecol 163:1430–1437, 1990.

72. Simon C, Frances A, Yon Lee B, et al.: Immunohistochemical localisation, identification and regulation of the IL 1 receptor antagonist in the human endometrium. Molc Hum Reprod 1:247-2477, 1995.

73. Simon C, Frances A, Piquette GN, Zurawsky G, Deng W, Polan ML: Interleukin 1 system in the maternal trophoblast unit in human implantation. Immunohistochemical evidence for autocrine/paracrine function. J Clin Endocrinol Metab 87:847–854, 1995.

74. Frank GR, Brar AK, Jirihara H, Cedars MI, Handweger S: Interleukin 1 beta and endometrium: an inhibitor of stromal cell differentiation and possible auto regulator of decidualisation in human. Biol Reprod 52:184–191, 1995.

75. Streelee GL, Currie WD, Leung E, Yuen BH, Leung PCK: Rapid stimulation of Human chorionic gonadotrophin secretion by interleukin 1 beta from perfused first trimester trophoblast. J Clin Endocrinol Metab 75:783–788, 1992.

76. Bananor RI, Piazza A, Rumi L, Polak de Fried E: Predictive value of interleukin 1 beta in supernatants of human embryo culture. Fertil Steril 80:407, 1992.

77. Sheth KV, Roca GL, Al Seidary ST, Parhar RS, Hamilton GJCM, Al-Abdul Jabbar F: Prediction of successful implantation by measuring interleukin 1 and immunosuppressive factor(s) in pre implantation embryo culture fluids. Fertil Steril 55:952–957, 1991.

78. Seifer DB, Romero R, Berlinsky D, Haning RV Jr.: Absence of cytokine production by pre implantation human embryo. Adv Res Inst 30:105–107, 1993.

79. Simon C, Mercader A, Gineno MJ, Pellicer A: The interleukin 1 system and human implantation. Am J Reprod Immunol 37:64–72, 1997.

80. Arii A, Engin O, Attar E, Olive D: Modulation of Leukemia Inhibitory Factor gene expression and protein biosynthesis in human endometrium. J Clin Endocrinol Metab 80:1908–1915, 1995.

81. Kojima K, Kanzaki H, Iwai M, et al.: Leukemia Inhibitory factor (LIF) gene expression in human endometrium. In: Nagata Y (ed): Proceedings of the 7th Annual Meeting of Japanese Society for Immunology of Reproduction. Kagoshima: pp. 245–248, 1993.

82. Kojima K, Kanzaki H, Iwai M, et al.: Expression of Leukemia Inhibitory Factor in human endometrium and placenta. Biol Reprod 50:882–887, 1994.

83. Stewart CL: A cytokine regulating embryo implantation. Second Conference on the Endometrium. Bologna. Abstract 93, 1993.
adhesion molecules by invasive cytotrophoblasts. J Clin Invest 91:950–960, 1993.
95. Yang Y, Yelavarthi KK, Chen HL, Pace JL, Terranova PL, Hunt JS. Molecular, biochemical and functional characteristics of tumor necrosis factor alpha produced by human placental cytotrophoblast cells. J Immunol 150:5613–5624, 1993.
96. Van de Stolpe A, Caldecoven E, Staele BG: 12-O-tetradecanoyl phorbol 13 acetate and tumor necrosis factor alpha mediated induction of intercellular adhesion molecule 1 is inhibited by dexamethasone. J Biol Chem 269:184–189, 1994.
97. Dailer E, Pampfer S, Yeung YG, Barad D, Stanley ER, Pollard JW: Expression of colony-stimulating factor-1 in the human uterus and placenta. J Clin Endocrinol. Metab 74:850–858, 1992.
98. Wu MC, Yunis AA: Common pattern of two distinct types of colony-stimulating factor in human tissues and cultured cells. J Clin Invest 65:772–775, 1980.
99. Kanzaki H, Yui J, Iwai M: The expression and localization of mRNA for colony-stimulating factor (CSF-1) in the human term placenta. Hum Reprod 7:563–567, 1992.
100. Pampfer S, Tahizhadeh S, Chuan FC, Pollard JW: Expression of colony-stimulating factor-1 (CSF-1) messenger RNA in human endometrial glands during the menstrual cycle; molecular cloning of a novel transcript that predicts a cell surface form of CSF-1. Mol Endocrinol 5:1931–1938, 1991.
101. Kariya M, Kanzaki H, Hanamura T: Progesterone-dependent secretion of macrophage colony-stimulating factor by human endometrial stromal cells of non-pregnant uterus in culture. J Clin Endocrinol Metab 79:86–90, 1994.
102. Shorter SC, Vince GS, Starkey PM: The identification of mRNA for the IL-3 related cytokines in placental and decidual tissues. In: Cedard L, Chaouat G, Challier JC, Neissmann C (eds): Proceedings of the Third Meeting of the European Placenta Group. Paris: INSERM. John Libbey Eurotext, p. 145, 1989.
103. Martal J, Assal NE, Assal Meliani A: Immuno endocrine properties of tau interferons in the maternal recognition of pregnancy. In: Glasser SR (eds): Endocrinology of Embryo Endometrium Interactions. Ciba Foundation Symposium. International Congress of Endocrinology, Bordeaux. Ciba Foundation Editors. New York: Plenum Press, pp. 195–216, 1993.
104. Bazer FW, Spencer TE, Ott TL: Interferons tau: a novel pregnancy recognition signal. Am J Reprod Immunol 1:42–49, 1997.
105. Fillion C, Chaouat G, Reinaud P, Charginy JC, Martal J: Immunoregulatory effects of trophoblastin (oTP): all 5 isoforms are immuno suppressive of PHA driven lymphocyte proliferation. J Reprod Immunol 19:237–249, 1991.
106. Assal-Meliani A, Charginy G, Reinaud P, Martal J, Chaouat G: Recombinant ovine trophoblastin (oTP) inhibits ovine, murine and human lymphocyte proliferation. J Reprod Immunol 25:148–165, 1993.
107. Roberts RM, Cross JC, Leaman DW: Interferons as hormones of pregnancy. Endocr Rev 13:432–452, 1992.
108. Chaouat G, Assal-Meliani A, Martal J: IL-10 prevents naturally occurring fetal loss in the CBA x DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by in vivo injection of IFN-t. J Immunol 154:4261–4268, 1995.
109. Whaley AE, Reddy Meka CS, Harbison LA, Hunt JS, Imakawa K: Identification and cellular localisation of unique interferon mRNA from human placenta. J Biol Chem 269:10864–10868, 1994.
110. DW, Hunter C, Mitchell MD, Warner MW, Gately M: Elevation of serum IL 12 levels in women with severe pre eclampsia and HELLP syndrome. J Dudley Reprod Immunol 31: 1–2, 97–108, 1996.
111. Sunder Plassman G, Derfler K, Wagner L: Increased serum activity of Interleukin 2 in patients with pre-eclampsia. J Autoimmun 2:203–205, 1989.
112. Boehm KD, Kelley MK, Iian J, Iian J: The interleukin 2 gene is expressed in the syncytiotrophoblast of the human placenta. Proc Nat Acad Sci 86:636–660, 1996.
113. Tezabwala BU, Johnstone AP: Effects of administration of interleukin 2 in pregnancy. J Reprod Immunol 147, 1986.
114. Lala PK, Parhar RS, Kearns M, Johnson S, Scodras JM: Immunological aspects of the decidual response. J Reprod Immunol (suppl)1:20, 1986.
115. Mattsson R, Holmdahl R, Scheinys A, Bernadotte F, Mattsson L: Placental MHC class I antigen expression is induced in mice following in vivo treatment with recombinant interferon gamma. J Reprod Immunol 19: 115–129, 1991.
116. Hunt JS, Hua Lin C, Miler L: Tumor necrosis factors: pivotal components of pregnancy? Biol Reprod 54:554–563, 1996.
117. Parand M, Chedid L: Protective effects of chlorpromazine against endotoxin induced abortion. Proc Soc Exp Biol Med 116:906–915, 1964.
118. Beaman KD, Hoversland RC: Induction of "spontaneous" abortion by blocking antigen specific suppression. J Reprod Fertil 82:135–139, 1988.
119. Hoversland RC, Beaman KD: Embryo implantation associated with increase in T cell suppressor factor in the uterus and spleen of mice. J Reprod Fertil 88:135–139, 1990.
120. Rubesa G, Beaman KD, Beer AE, Haller H, Rukavina D: Expression of membrane associated protein T]6 on decidual lymphocytes in the first trimester of pregnancy. J Reprod Immunol 30:12–27, 1996.
121. Beaman K, Angkachatchai V, Gillman Sachs A: T]6, the pregnancy associated cytokine. Am J Reprod Immunol 35:338–342, 1996.
122. Stekerees Bartho J: Immunosuppression by Progesterone in Pregnancy. Boca Raton: CRC Press, 1992.
123. Stekerees Bartho J, Wegmann TG: A progesterone dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol 31:81–97, 1996.
124. Stekerees Bartho J, Faust P, Varga P, Szereday L, Kelle-
men K: The immunological pregnancy protective effect of progesterone is manifested via control of cytokine production. Am J Reprod Immunol 35:348–352, 1996.

125. Check JH, Arwitz M, Gross J, Szekeres Bartho J, Wu CH: Evidence that expression of progesterone induced blocking factor by maternal T lymphocytes is positively correlated with conception. Am J Reprod Immunol 38:6–9, 1997.

126. Leah RG, Vince G, Flanders KC, et al.: Uterine TGF beta 2 in human normal and pathologic pregnancies. In: Early Embryo Development, Uterus Preparation and Role of Cytokines in Implantation and Labour. Lyon: Editions Fondation Marcel Mérieux, pp., 1995.

127. Wegmann TG, Lin H, Guilbert L, Mossman TH: Bidirectional cytokine interactions in the maternal-fetal relationship: successful allogenesis is a Th2 phenomenon. Immunol Today 14:353–355, 1993.

128. Bennet WA, Lagoo-Dennayan S, Whitworth NS, Brackin MN, Hale E, Cowan BD: Expression and production of interleukin 10 by human trophoblast: a model for cytokine regulation of pregnancy immunotolerance. In: The Third World Conference on Early Pregnancy. New York: Parthenon Publishing, p. 75, 1996.

129. Roth I, Corry D, Locksley R, Abrams J, Litton M, Fisher S: Human placental cytrophoblasts produce the immunosuppressive cytokine interleukin 10. J Exp Med 184:539–548, 1996.

130. Mincheva Nillson L, Baranov V, Hammarstrom ML, Hammarstrom S: Phenotypic and functional analysis of human decidua associated lymphoid cells from normal human pregnancy. In: Early Embryo Development, Uterus Preparation and Role of Cytokines in Implantation and Labour. Lyon: Editions Fondation Marcel Mérieux, pp. 113–141, 1995.

131. Delassus S, Countinho GS, Sauzier S, Darthe S, Kourilky P: Differential cytokine expression in maternal blood and placenta during murine gestation. J Immunol 152:2411–2420, 1994.

132. Lin H, Mossman TR, Guilbert L, Tuntipopipat S, Wegmann TG: Synthesis of THelper-2 cytokines at the maternal-fetal interface. J Immunol 151:4562, 1993.

133. Krishnan L, Guilbert LJ, Wegmann TG, Belosevic M, and Mossman TR: THelper-1 response against Leishmania major in pregnant C57Bl/6 mice increases implantation failure and fetal resorptions. Correlation with increased IFN-g and TNF and reduced IL-10 production by placental units. J Immunol 156:653, 1996.

134. Krishnan L, Guilbert LJ, Russell AS, Wegmann TG, Mossman TR, Belosevic M: Pregnancy impairs resistance of C57Bl/6 mice to Leishmania major infection and causes increased antigen-specific IFN-g response and increased production of THelper 2 cytokines. J Immunol 156:6494, 1996.

135. Tangri S, Raghupathy R: Expression of cytokines in placentas of mice undergoing immunologically mediated spontaneous foetal resorptions. Biol Reprod 49: 850–856, 1993.

136. Tangri S, Wegmann TG, Lin H, Raghupathy R: Maternal anti placental activity in natural, immunologically mediated fetal resorption. J Immunol 152:4903–4910, 1994.

137. Hill J: THelper 1 immunity to trophoblast: evidence for a new immunological mechanism for recurrent abortion in women Hum Reprod 10:114–120, 1995.

138. Hill JA, Polgar K, Anderson DJ: Helper TH1 immunity in women with recurrent spontaneous abortion. JAMA 273:1993–1935, 1995.

139. Guimond MJ, Luross JA, Wang B, Terhorst C, Danial S, Croy BA: Absence of natural killer cells during murine pregnancy is associated with reproductive compromise in TgE26 mice. Biol Reprod 56:169–17.

140. Chaouat G, Tranchot Diallo J, Volumenche JL, et al.: Immune suppression and TH1/TH2 balance in pregnancy revisited. A (very) personal tribute to Tom Wegman. Am J Reprod Immunol 37:427–435.

141. Chaouat G, Clark DA, Wegmann TG: Genetics aspects of the CBAxJ x DBA/2 J and B10 x B10.A models of murine spontaneous abortions and prevention by leukocyte immunisation. In: RCOG, Allen WR, Clark DA, Gill TJ III, Mowbray JF, Robertson WR (eds.): 18 th RCOG Study Group. Early Pregnancy Loss. Mechanisms and Treatment. RCOG Press, pp. 89–105, 1998.

142. Kinsky R, Delage G, Rosin N, Thang MN, Hoffmann M, Chaouat G. A murine model of NK mediated foetal resorption. Am J Reprod Immunol 23:73–78, 1990.

143. Chaouat G, Menu E, David F, Dijan V, Kinsky R: Reproductive immunoLOGY 1989–1992: some important recent advances about fetomaternal relationship. In: Gergely J (ed.) Progress in Immunology 8 (8th International Congress Immunology) Springer Verlag, p. 825, 1993.

144. Kinsky R, Kapovic M, Menu E: The role of defined major histocompatibility antigens in preventing foetal death. In: The Immunology of Pregnancy. Boca Raton: CRC Press, pp. 47–61, 1993.

145. Chaouat G: Synergy of lipopolysaccharide and inflammatory cytokines in murine pregnancy: alloimmunization prevents abortion but does not affect the induction of preterm delivery. Cell Immunol 157:328, 1994.

146. Gendron RL, Nestel FP, Lapp WS, Baines MG: Lipopolysaccharide induced fetal resorption in mice is associated with the intruterine production of tumor necrosis factor-a. J Reprod Fertil 90:447, 1990.

147. Duclos AJ, Haddad EK, Baines MG: Presence of activated macrophages in a murine model of early embryonic loss. Am J Reprod Immunol 33:354, 1995.

148. Haddad EKA, Duclos J, Lapp WS, Baines MG: Early embryonic loss is associated with the prior expression of macrophage activation markers in the decidua. J Immunol 158:4886, 1997.

149. Haddad EK, Duclos AJ, Baines MG: Early embryonic loss is associated with local production of nitric oxide by activated macrophages. J Exp Med 182:1143–1152, 1995.
150. Baines MG, Duclos AG, Antecka E, Haddad EK: Decidu- 
al infection and activation of macrophages lead to early embryo loss. Am J Reprod Immunol 37:471- 
478, 1997.

151. De Fougerolles R, Baines M: Modulation of Natural 
Killer activity influences resorption rates in CBA x 
DBA/2 matings. J Reprod Immunol 11:147, 1988.

152. Gendron R, Baines M: Infiltrating decidual Natural 
Killer cells are associated with spontaneous abortion in 
mice. Cell Immunol, 113:261, 1988.

153. Chaouat G, Menu E, Wegmann T: Role des lympho-
kines de la famille du CSF, et du TNF, de l'intérfon 
gamma, et du IL-2 sur la survie fetale et la croissance 
placentaire étudiées in vivo dans 2 modèles 
avortements immunitaires spontanés murins. Biolo-
gie cellulaire et moléculaire de la relation materno fe-
tale. Paris: Editions INSERM John Libbey, pp. 91-
101, 1991.

154. Chaouat G, Menu E, Wegmann T: Role des lympho-
kines de la famille du CSF, et du TNF, de l'intérfon 
gamma, et du IL-2 sur la survie fetale et la croissance 
placentaire étudiées in vivo dans 2 modèles 
avortements immunitaires spontanés murins. Biolo-
gie cellulaire et moléculaire de la relation materno fe-
tale. Editions INSERM John Libbey: Pages 91–101, 
1991.

155. Clark DA, Chaouat G, Arck P, Merali F, Hoskin D, 
etal.: Murine pregnancy decidualizing TNF-a and IL-1 block stress-triggered mu-
rine abortion. Am J Reprod Immunol 37:262–267, 
1997.

156. Clark DA, Arck P, Jalali R, et al.: Psychoneurocytokin 
endocrine pathway in immunoregulation during preg-
nancy. Am J Reprod Immunol 33:330–337, 1996.

157. Arck P, Merali F, Stead R, Chaouat G, Clark DA: 
Stress triggered abortion: inhibition of protective sup-
pressor mechanisms and promotion of TNF alpha re-
lease via neuro transmitter. Am J Reprod Immunol 
33:74–80, 1995.

158. Markert U, Azrek CP, McBey BA, et al.: Stress trig-
gered abortion are associated with alterations of granu-
lated cells in the decidua. Am J Reprod Immunol 37: 
94–101, 1997.

159. Markert U, Azrek CP, McBey BA, et al.: Stress trig-
gered abortion in mice is mediated by the procoagulant 
fgl2 prothrombinase. In press. Stoppacciaro A, Melani C, 
Parenz A, et al.: Regression of an established tumor geneti-
ically modified to release granulocyte colony-stimulating fac-
tor requires granulocyte-T cell cooperation and T cell-
produced interferon g. J Exp Med 178:151, 1997.

160. Markert U, Azrek CP, McBey BA, et al.: Stress trig-
gered abortion in mice is mediated by the procoagulant 
fgl2 prothrombinase. In press. Stoppacciaro A, Melani C, 
Parenz A, et al.: Regression of an established tumor geneti-
ically modified to release granulocyte colony-stimulating fac-
tor requires granulocyte-T cell cooperation and T cell-
produced interferon g. J Exp Med 178:151, 1997.

161. Arck P, Merali C, Chaouat G, Clark DA: Inhibition of 
immunoprotective CD8+ cells as a basis for stress 
triggered abortion in mice. Substance P mediated abor-
tion in mice. Cell Immunol 171:226–230, 1996.

162. Clark DA, Arck P, Jalali R, et al.: Psycho neuro cytotoxic 
endoctrine pathway in immunoregulation during preg-
nancy. Am J Reprod Immunol 33:330–337, 1996.

163. Arck P, Merali F, Stead R, Chaouat G, Clark DA: 
Stress triggered abortion: inhibition of protective sup-
pressor mechanisms and promotion of TNF alpha re-
lease via neuro transmitter. Am J Reprod Immunol 
33:74–80, 1995.

164. Markert U, Azrek CP, McBey BA, et al.: Stress trig-
gered abortion in mice is mediated by the procoagulant 
fgl2 prothrombinase. In press. Stoppacciaro A, Melani C, 
Parenz A, et al.: Regression of an established tumor geneti-
ically modified to release granulocyte colony-stimulating fac-
tor requires granulocyte-T cell cooperation and T cell-
produced interferon g. J Exp Med 178:151, 1997.
176. Menu E, Kaplan L, Andreu G, Denver L, Chaouat G: Immunoactive products of human placenta, I. An immunoregulatory factor obtained from explant cultures of human placenta inhibits CTL generation and cytotoxic effector activity. Cell Immunol 119:341, 1989.

177. Menu E, Jankovic DL, Theze J, David V, Chaouat G: Immunoactive products of human placenta, III. Characterization of an inhibitor affecting lymphocyte proliferation. Regional Immunol 3:254–259, 1991.

178. Menu E, Kinsky R, Hoffman M, Chaouat G: Immunoactive products of human placenta, IV. Immunoregulatory factors obtained from cultures of human placenta inhibit local and general murine graft versus host reactions (GVHR). J Reprod Immunol 20:195–204, 1991.

179. Menu E, Djian V, Kinsky R: Immunoactive products of human placenta. Biologie cellulaire et moléculaire de la relation materno-fetale. Paris: Editions INSERM John Libbey, pp. 197–203, 1991.

180. Djian V, Menu E, Thibault G, Ropert S, Chaouat G: Immunoactive products of placenta. V. Immunoregulatory properties of a low molecular weight compound obtained from human placental cultures. Am J Reprod Immunol 36:11–24, 1996.

181. De Smedt D, Menu E, Chaouat G. Immunoactive products of placenta VI. Induction of T cell anergy by a low molecular weight compound obtained from supernatants of human placental cultures. Cell Immunol 175:128–140.

182. Voluménie J-L, Mognetti B, De Smedt D, Menu E, Chaouat G. Induction of transient murine T cell anergy by a low molecular weight compound obtained from supernatants of human placental cultures is linked to defective phosphorylation of TcR ξ CD3 chain. Am J Reprod Immunol. In press.

183. Migita K, Eguchi K, Kawabe Y, Tsukada T, Ichinose Y, Nagataki: Defective TcR chain phosphorylation in SEB induced anergic T cells. J Immunol 15:5083–5089, 1995.

184. Mincheva-Nilsson L, Hammarström S, Hammarström ML: Human decidual leukocytes from early pregnancy contain high number of gamma-delta+ cells and show selective down regulation of alloreactivity. J Immunol 149:2203, 1992.

185. Baines MG, Gendron RL: Natural and experimental animal models of reproductive failure in Immunology of Pregnancy. In: Chaouat G (ed.) Boca Raton, Florida: CRC Press. 173–203, 1993.