# Reproductive Immunology and the Placental Barrier Hypothesis*

**Phillip Gambel, Ph.D.**
**Rosemarie D. Hunziker, M.Sc.**
**Thomas G. Wegmann, Ph.D.**

Previous studies in this laboratory have indicated that the placenta can serve as an immunoabsorbent barrier for monoclonal antibodies directed against Class I major histocompatibility complex (MHC) antigens of paternal strain origin.[1-4] When these antibodies bind to the placenta, they are internalised and degraded by trophoblast cells, and the fragments eventually appear in the maternal serum.[5] From these studies, we have concluded that the placenta can serve as a barrier to potentially damaging antibodies that would otherwise gain access to the foetus. More recently, we have extended our studies to the placenta as a barrier to maternally-derived cells. By using either genetic markers or fluoresceinated cells, we have been able to show that the placenta is a selective cell barrier. Small numbers of maternal red cells can routinely cross the placenta, but the transit of maternal lymphocytes into the foetus is usually prevented.[6] We have also recently used antibody-facilitated chimeras to determine that most of the cells of the decidua are not bone marrow-derived, in contrast to the conclusions suggested by other investigators.[7] A third area discussed is the development of an animal model of prevention of spontaneous abortion by vaccination with allogeneic cells. This might lead to new insight into the way the placenta protects the foetus.

### The placenta as an immunoabsorbent barrier

The first issue that my colleagues and I have addressed is the expression of H-2 antigens in the placenta. The approach has been to inject radiolabelled polyclonal,[1] and in subsequent studies monoclonal,[2-4] antibodies into the circulation of the mother to determine where they bind and, ultimately, their fate. The conclusion from these studies is that the placenta can indeed serve as an immunoabsorbent sink, binding antibodies directed against Class I paternal MHC antigens (but not Class II MHC antigens) and preventing their access to the foetal circulation. More recent studies indicate that when these antibodies bind to the surface of placental cells, a certain amount is internalised, digested, and released back into the maternal circulation as fragments.[5] This binding and digestion only occurs if the foetus bears the target antigen. These results, taken as a whole, confirm and extend the results of others in the mouse. A recent publication supports the idea of the placenta as an immunoabsorbent barrier in a natural setting *in vivo*, using pregnancy-induced polyclonal antibody, while questioning whether the barrier is 100 per cent (versus 97%) efficient.[8] However, the use of foetally-derived fibroblasts to detect antibody in foetal serum in this study raises questions concerning the specificity of the antibody being detected. Perhaps the most interesting conclusion from our placental studies is that Class I MHC antigens are directly exposed in the spongiotrophoblast to cells of maternal origin,[9] thus eliminating a number of possible explanations of why the foetal-placental unit is a privileged exception to the usual rules of allogeneic transplantation. Indeed, T.J. Gill III and his associates have found that the only detectable humoral immune response of rat females during pregnancy is to Class I MHC antigens.[10] Also, Class I HLA antigens have recently been found on cytotrophoblast in the human placenta.[11]

### The origin of decidual cells

While attention will continue to be focussed on the role/fate of anti-

*From the Department of Immunology and MRC Group on Immunoregulation, 8-45E Medical Sciences Building, The University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

bodies bound to the placenta, this laboratory is establishing methods to study maternal immune effector cells of the placenta and the surrounding tissue. One stimulus to our current efforts was provided by a report which suggested that most cells of the decidua might be ultimately derived from bone marrow stem cells.[7] This was based on the use of radiation chimeras in which a parental animal was repopulated with $F_1$ hybrid bone marrow. Although pregnancy in such an animal is impossible, pseudopregnancy can be induced by hormones and by injecting oil into the uterus leading to a deciduoma. When this deciduoma is processed for cells suitable for H-2 typing, the cells are found to be substantially of donor origin. Experiments reported at the International Conference of Reproductive Immunology – held in Kyoto, Japan, in August of this year – using human decidua are based on the assumption that the interpretation from the mouse experiments is correct, and can be applied to the human case where lymphoid-like cells can be detected by mAb on frozen placental sections.[11] We have re-examined this question using a different approach. We can construct $F_1$ hybrid female mice with virtually 100 per cent of their bone marrow derived from a parental source AFCh, by using monoclonal anti-host MHC antibody treatment as a way of preparing the bone marrow bed for the graft.[12] Since these mice are produced without irradiation, they are not sterile, and can become pregnant after repopulation. When examined at day 7½ of gestation by the glucose phosphate isomerase isozyme (GPI) assay to determine chimerism – which can be done using whole tissue – the majority of the decidua is host, and not bone marrow donor, in origin. We have found that the same is true for oil-induced granulomas in radiation chimeras such as those employed by Kearns and Lala. However, when we do a GPI analysis of decidual cells prepared with collagenase, a technique generally employed to isolate decidual cells, we find a substantial percentage of donor contribution, *albeit* reflecting only a minority of cell components of the whole decidua tissue. These isolated cells also display natural killer (NK) activity (as assayed in a 4-hour $^{51}Cr$ release of labelled YAC targets). The NK activity can be partially abrogated by treatment with anti-asialo GM1 or anti-Thy 1.2 in the presence of complement. We therefore conclude that the bulk of the decidua is host derived from stromal cells, but the decidua does contain a minority cell population which is bone marrow-derived, and is capable of natural killer activity. It is our belief that the decidua is made up of heterogeneous populations which change over the course of gestation.

### The placenta as a cell barrier

Historically, there have been a number of claims that maternal cells can traverse the maternal-foetal barrier and enter the foetus (reviewed in 13, 14, 15; see also 16, 17). As another approach to our studies on the placenta as a barrier to cells, we decided to examine this question directly in two ways. One is by using naturally occuring markers – those of the GPI system – to evaluate maternal cell trafficking into the foetus and *vice versa*. We could find no trafficking in either direction, but this assay is only sensitive at the 1 per cent level of detection. We therefore decided to improve the sensitivity by injecting large numbers of fluoresceinated red and white cells into the maternal circulation using the method of Butcher and Weissmann.[18] In 11 out of 30 foetuses, red cells were found to traverse the placenta in low numbers that varied from foetus to foetus. White cells, however, did not seem to breach the barrier (with one exception out of 24 foetuses), since we found no more than 225 labelled cells in a fetal liver.

### Prevention of spontaneous abortion in mice

If the field of reproductive immunology is to progress to the level of understanding how the placenta prevents maternal cell-mediated immunity from harming the allogeneic conceptus *in situ*, situations must be found in which immunological manipulations can influence fetal survival, and the basis for this influence can be further analysed into its component parts. A start in this direction has been made in human pregnancy, where recurrent spontaneous abortion can be prevented by vaccination either with the husband's white cells or with pooled white cells from a variety of donors.[10,19–21] These reports followed, in part, from observations in humans that serum blocking factors were absent or reduced in the serum of women undergoing spontaneous abortion, and the apparent success of such procedures seemed to justify belief in the importance of such factors. Dr Alan Beer, whose group has been one of the leaders in this area, is now urging caution with respect to this procedure (Beer, A., personal communication, 1983). The first patient born using this treatment in Beer's series has now come down with what appears to be severe combined immunodeficiency disease (SCID) at 18 months of age. The patient is currently being evaluated at the Sloan-Kettering Institute for the presence of maternal cells in his circulation. The graft-versus-host disease commonly seen in such SCID patients is presumably due to maternal lymphocytes that cross the placenta.[17] Here, humans studies of course, suffer from the inability to dissect the mechanisms involved. An animal model is needed to put this procedure on a more rational footing and provide an explanation for how the effect is mediated. Such a model now exists. It is based on the observations of D. Clark and his associates, who showed that CBA females pregnant by DBA give rise to a high

incidence of spontaneous abortion, and this correlates with the lack of suppressor cells in the vicinity of the foetal-placental unit.[22] The essence of the model is that vaccination by BALB/c (but not DBA or CBA) spleen cells one week prior to mating abrogates the spontaneous abortion (from 23% to 5%, $p < 0.001$).[23] More recent studies indicate that such vaccination increases the level of active suppression against natural killer cells in the placenta itself and also leads to an increase in anti-paternal H-2 antibodies, primarily of the IgG1 non-complement binding isotype. These IgG1 antibodies are differentially absorbed from the serum in the pregnant females, presumably on the placenta (Chaouat, G., *et al.*[24] Taken together, these observations continue to point to the importance of the trophoblast barrier not only a passive anatomic barrier but an active intervening tissue as well. As such, this model has both basic and clinical implications.

In conclusion, the placenta can serve as a barrier to both antibodies and cells of potential danger to the foetus. The exact mechanisms that mediate this effect are under intense scrutiny and should be resolved in the near future.

## Summary

We describe experiments which demonstrate that the placenta serves as a specific immunoabsorbent barrier for antipaternal MHC Class I antibodies. The placenta also serves as a selective barrier to maternal white blood cells, while allowing red blood cells across. In order to further understand the roles of the placenta as a barrier to maternal immunity, animal models in which vaccination leads to increased foetal viability are required. A murine model with these properties is described, along with some of the immunological changes that accompany the vaccination.

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