

Maternal Allo-recognition of the Fetus

Ashley Moffett^{1,3*} Olympe Chazara^{1,3} & Francesco Colucci^{2,3}

¹Dept of Pathology and ²Dept. of Obstetrics & Gynaecology, and ³Centre for Trophoblast Research, University of Cambridge, UK

*Corresponding Author:

Prof. Ashley Moffett

am485@cam.ac.uk

+44 1223 333727

Introduction

Since the original paper by Peter Medawar in 1953, the question that has dominated the field of reproductive immunology is: “Why is the fetus not rejected?” This question arose because Medawar (who had begun working on transplantation of skin grafts during WWII to treat pilots with burn injuries) showed that graft rejection is an immunological phenomenon (1,2). His essay raised the question of why in pregnancy it is possible for two genetically different individuals to coexist without rejection of the fetus. We describe here the immunological basis of allo-recognition and how this relates to human pregnancy.

Anatomy of Maternal/Fetal Interaction

The implanting blastocyst is surrounded by trophoctoderm that will develop into the definitive villous placenta as well as the invading extravillous trophoblast cells that invade into the uterus to tap into the maternal blood supply. Villous trophoblast is in contact with maternal blood circulating through the intervillous space. Syncytial ‘knots’ are also shed from the villous placenta directly into the systemic circulation and become entrapped in the lung capillaries. EVT comes into direct contact with tissue immune cells in the decidua and myometrium of the uterus. Thus, fetal somatic cells are normally completely separated from the maternal immune system by the placental trophoblast barrier (3).

Fetal cells can, however, cross over into the maternal circulation. This usually occurs during the trauma at delivery (although they can also cross during spontaneous or therapeutic abortions) and these cells are capable of initiating immune responses. In the early days of transplantation it was noted that multiparous women had antibodies to allogeneic leukocytes (allo-antibodies), now known to be specific for paternal Human leukocyte Antigens (HLA) (4). The presence of these allo-antibodies has no influence on the outcome of pregnancy but will affect the mother’s chances of finding a compatible organ donor. The presence of anti-D antibodies in women who are negative for this

blood group antigen also reveals that pregnant women can generate antibodies to fetal allo-antigens and they are therefore not immunosuppressed (5).

Therefore, maternal immune responses in pregnancy should be considered separately as:

- Systemic responses to fetal cells or soluble fetal antigens
- Systemic responses to villous syncytiotrophoblast
- Uterine immune responses to EVT.

T cell allo-recognition

The cells capable of allo-recognition are lymphocytes: T cells, Natural Killer (NK) cells, and B cells that produce antibodies with T cell help (6).

T cells have a clonally-distributed receptor (TCR) that is generated during fetal development by somatic gene rearrangement. Any T cells that have a TCR specific for a self HLA molecule presenting a self peptide are eliminated during T cell development to avoid self-reactivity (central tolerance). Allo-reactive T cells will be present amongst the huge diversity of an individual's T cells at a frequency of <10%. These T cells will be able to respond and attack organ grafts unless there is HLA matching of donor and recipient.

Rejection of solid organ grafts results from stimulation of the recipient's immune system by two pathways: direct and indirect allo-recognition (6). In direct allo-recognition, donor dendritic cells, antigen presenting cells (APC) that express donor HLA molecules, migrate from the graft to the draining lymph node respectively. HLA class I and class II molecules bind directly to recipient's CD8+ and CD4+ T cells in the lymph node. Effector T cells move into the graft with CD8+ T cells killing the graft and CD4+ T cells activating macrophages to initiate inflammation and providing help to B cells to produce antibodies. Indirect allo-recognition results from uptake of HLA and other allo-antigens from donor's cells by recipient APC that drain to the regional lymph node. Peptides derived from the donor's HLA molecules are then presented to recipient's T cells initiating a CD4+ allo-reaction.

Why do maternal allo-reactive T cells not attack the fetal trophoblast? Several mechanisms have been described in both humans and mice. The villous syncytiotrophoblast never expresses any HLA class I or class II molecules so it is not possible for any T cells to bind to the main placental barrier, clearly a highly effective mechanism to protect the placenta from being killed (7). The syncytial nature of the placental barrier is also likely to be important as small defects in the membrane can heal rapidly. In contrast, the EVT does express HLA class I but never class II molecules so it cannot act as an APC initiating direct allo-recognition to CD4+ T cells.

Furthermore, the set of HLA class I molecules expressed is unusual: HLA-C, HLA-G and HLA-E. Of these only HLA-C is polymorphic and so the paternal allele donated to the fetus will differ from the mothers. HLA-G is monomorphic and is unique amongst HLA class I molecules as it forms a homodimer that can bind with high avidity to LILRB1, an inhibitory receptor expressed by all decidual APC. This interaction probably deviates immune responses towards a tolerogenic rather than an immunogenic response (8). This has the added benefit of only occurring when there is direct physical contact between HLA-G+ EVT and uterine APC allowing maternal APC elsewhere in her body to function normally.

Regulatory T cells (Tregs) can suppress allo-reactive CD4+ and CD8+ T cells and are generated in the decidua in pregnancy in both humans and mice probably because of the unique microenvironment rich in factors such as TGF (9). However, it is still not clear whether the TCRs of these Tregs have any specificity for trophoblast HLA-C class I molecules although one study analysing T cells in the decidua at term has shown there are increased percentages of functional Tregs in HLA-C mismatched pregnancies (10). Other mechanisms described in murine models include reduced migration of APC to draining lymph nodes, failure of effector T cell accumulation in the decidua by silencing of stromal cell-derived chemokines and global effects of progesterone on immune cells (11). Mouse models may not be as helpful in studying reproductive failure as they have been for analysing the immunological basis of other human diseases. Pregnancy in the mouse only last 19 days, there is no menstrual cycle, and the anatomy of placentation is different with little trophoblast invasion and formation of decidua only triggered by implantation and not in the secretory phase of the pre-pregnant endometrium.

Although women in pregnancy are not immunosuppressed as seen by responses to paternal HLA and other allo-antigens, they do respond differently to infectious agents and auto-antigens. For example, they are particularly susceptible to influenza, chicken pox and other viruses and the severity of auto-immune disorders such as rheumatoid arthritis and multiple sclerosis varies during pregnancy (12,13). This may be due to a deviation towards making better Th1 type responses (cytotoxic and effective for viruses) than antibody-generating Th2 type responses in pregnancy although robust evidence for this in humans is still lacking. These alterations in the shape of immune responses in pregnant women are likely to result from enormous changes. Although these systemic changes in the type of immune responses seen in pregnancy mean, for example, that women need vaccination for influenza virus, it is important to state that there is still no evidence they have any impact on reproductive success and are likely to be an epiphenomenon secondary to the high levels of progesterone and other hormones and placental products.

The important unanswered question is whether in humans T cells ever do bind and attack the trophoblast cells resulting in pregnancy failure? Placental mammals evolved ~150 million years ago and there must have been strong selective pressures to avoid T

cell rejection of the fetus. Because there are multiple mechanisms already described to avoid effector T cell responses that might damage trophoblast cells it seems unlikely that will ever all fail together. Indeed, there are no convincing reports in humans that this does happen and that maternal T cells with specificity for trophoblast have caused killing of the placental cells. Perhaps it is time to move away from Medawar's famous question of 1953 and view the co-existence of the mother and her baby not as a dichotomy between rejection and acceptance but as a compromise.

NK cells

Although NK cells were only discovered in the 1970s, in evolutionary terms they are much older than T cells (14). Equivalent cytotoxic cells are present in invertebrates whereas T cells only appeared in teleost fishes. NK cells resemble CD8⁺ T cells in many phenotypic, functional and morphological respects but crucially they lack a TCR generated by somatic gene rearrangement and rely on germ cell encoded receptors for target cell recognition. NK cells are particularly important in the early stages of viral infection and cancer. They were originally thought to be able to kill cells independently of HLA class I molecules but it then became clear that they are inhibited by binding to self-HLA and will therefore kill cells that lack self HLA class I molecules – known as the missing self response (15). Thus, CD8⁺ T cells kill cells expressing non-self HLA whilst NK cells kill cells lacking self HLA class I molecules; either the detection of a difference (T cells) or the absence of a similarity (NK cells). The findings that NK cells are the dominant lymphoid cells (~50-70% leukocytes) present at the time the placenta implants and become established led to the idea that they are the cells capable of allo-recognition the fetal EVT (16). T cells are present in early decidua but only account for ~5-20% of leukocytes. The remaining cells are CD14⁺ macrophages together with a few dendritic cells. The observation that B cells and plasma cells are rare means it is highly unlikely that antibodies to EVT are locally generated.

Uterine NK cells (uNK) are phenotypically distinct from those circulating in blood; they are CD56^{superbright}, CD16⁻ whereas 90% of blood NK cells are CD56^{dim}, CD16⁺ (17). The minor CD56^{bright} population in blood also differ from those in the uterus morphologically and for many other surface markers. These differences mean that measurements of any NK parameters in NK cells from blood will not be informative in relation to function of uNK (18). Uterine NK cells proliferate and differentiate in the mucosa and may originate from a resident progenitor cell or from a progenitor cell recruited from the blood. They are not present pre-menarche or post-menopausally and their dependence on the ovarian hormone, progesterone, is obvious from the great surge in proliferative activity after ovulation. This is mediated by increased expression of IL-15 from stromal cells in response to progesterone. The presence of uNK in the decidua is maximal in the first trimester and numbers thereafter fall so although they are still present at term they are not abundant (19). The functions of uNK are essentially unknown but available evidence suggests that they play a role in regulating

placentation and access of EVT to the spiral arteries that are the feto-placental supply line.

NK allo-recognition

NK cells are important clinically in haematopoietic transplantation. HLA matching between donor and recipient prevent the problem of graft-versus-host-reaction (GVHR) when donor T cells attack recipient's cells. Haploidentical transplants are used when no HLA-matched donors can be found for the patient, as most will have a family member who will share one of their two HLA haplotypes (20). The grafts need to be depleted of T cells to prevent GVHR and after engraftment donor NK cells will reconstitute rapidly in the recipient. These donor NK cells can be highly effective in recognising allogeneic recipient cells and create a beneficial graft-versus-leukaemia (GVL) effect (21).

Whether GVL occurs depends on receptors expressed by NK cells known as killer immunoglobulin-like receptors (KIR) that are germ-line encoded (22). The KIR gene family is highly variable between individuals both in the number of KIR genes inherited and additionally with allelic variability at each KIR locus. KIR genes can either impart an inhibitory or an activating signal to the NK cell and its overall functional response depends on the balance between the inhibitory and activating input to the cell following target recognition. The extent of KIR polymorphism is as great as for HLA genes and these two gene families are far more variable than any others in the human genome with large differences in frequency of variants in different populations. Some KIR expressed by NK cells bind to some groups of HLA-B (Bw4) and HLA-A (A3 and A11) class I molecules, but the dominant ligands for KIR are HLA-C molecules (22). The best scenario for effective GVL is if the recipient's residual leukaemic cells lack an HLA-C ligand for an inhibitory KIR expressed by donor's engrafting NK cells – missing self. In this situation the donor NK cells are not inhibited by recipient HLA-C and will kill the leukaemic cells (23).

Maternal NK recognition of fetus

NK allo-recongnition occurs in the placental bed in the uterus in a physiological not iatrogenic context. The mother inherits a particular set of KIR genes, two KIR haplotypes, from her parents. Broadly speaking these can be classified as KIR A or KIR B haplotypes. KIR A haplotypes only have inhibitory KIR but KIR B haplotypes have varying numbers of additional mainly activating KIR. Because the only HLA KIR ligand expressed by EVT are HLA-C molecules and these are the only polymorphic trophoblast HLA molecules, they provide a paternal HLA-C ligand for KIR expressed by maternal uNK. All the alleles of HLA-C can be divided into 2 groups, C1+HLA-C and C2+HLA-C, depending on the sequence of the KIR-binding site of the HLA molecules (KIR epitope). The possible combinations of maternal NK KIR interactions with fetal EVT HLA-C groups are illustrated (Fig. 1). Genetic studies show that the women who are most at risk of disorders of pregnancy associated with defective placentation (pre-eclampsia, unexplained stillbirth, fetal growth restriction) are more likely to have two KIR A

haplotypes with a fetal C2+HLA-C derived from the father (24,25). How this translates into different functions of uterine NK cells compared to pregnancies with different maternal KIR/fetal HLA-C combinations is under intense investigation. There is a very strong inhibitory signal imparted to uNK cells following binding of inhibitory KIR to C2+HLA-C molecules.

This type of allo-recognition based on NK cells is only operating in a normal physiological situation during early pregnancy when the placenta implants. The hypothesis we are investigating is that NK allo-recognition system defines the territorial boundary between the two genetically different individuals during placentation. This allows controlled invasion during placentation so the arteries are effectively transformed by trophoblast without undue penetration of the uterus. This border control is needed to balance the allocation of resources between mother and baby so that both can survive and flourish; thus, cooperative allo-recognition not leading to rejection or killing.

Figure Legend

Figure 1. Possible interactions between maternal KIR and fetal HLA-C molecules depend on *KIR* and *HLA-C* variants respectively present in the mother and fetus. Women can have inhibitory or activating KIR for which there is no HLA-C ligand in the fetus. The genetic combination of a mother with two *KIR A* haplotypes with a fetus carrying a group C2+*HLA-C* allele is associated with increased risk of pre-eclampsia. There is a very strong inhibitory signal from a fetal C2+HLA-C when maternal NK cells lack activating KIR (not present on *KIR A* haplotype). There is a protective effect for pre-eclampsia when a mother has an activating KIR for C2+ HLA-C that is found on *KIR B* haplotypes. Adapted from (26).

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