REVIEW

Placental immunology and maternal alloimmune responses

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Vox Sanguinis

During pregnancy, women are tolerant of their semi-allogeneic fetus whilst not being immunosuppressed and indeed readily form alloantibodies. This 'Immunological Paradox of Pregnancy' may be explained by an understanding of placental anatomy and immunology. Trophoblast cells form the interface between the fetus and maternal tissues and blood and escape allorecognition because they lack classical human leucocyte antigen (HLA) class I and II molecules. Local immunoregulation, or tolerance, in the decidua is mediated partly by HLA-G$^+$ extravillous trophoblasts (EVT) that invade the tissue and prevent killing by maternal natural killer cells, cytotoxic T cells and macrophages. Placental hormones orchestrate the composition and regulatory function of maternal immune cells. In contrast, syncytiotrophoblast cells at the surface of chorionic villi, in contact with maternal blood, maintain a state of mild maternal systemic immunity via activation of innate immunity and skewing towards humoral immunity. This enables maintenance of a healthy immune system in pregnant women and robust protective antibody responses to pathogens whilst enabling survival of the fetus. However, this has the unfortunate consequence that pregnant women readily form alloantibodies to incompatible alloantigens on fetal red cells, platelets and leucocytes if fetomaternal haemorrhage (FMH) occurs. The antibodies are initially low affinity but after re-immunization with further FMH become functionally effective, high-titre IgG.

Key words: fetomaternal haemorrhage, IgG, Immunology, placenta, platelets, pregnancy.

Introduction

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the platelet 'equivalent' of haemolytic disease of the fetus and newborn (HDFN). Differences in the development of alloantibodies to platelets or red cells may indicate dissimilar aetiologies. Approximately 80% of cases of FNAIT are caused by antibodies to the human platelet antigen (HPA)-1a [1], but this alloantibody is rarely formed after transfusion [2, 3]. Anti-HPA-5b and anti-HPA-1b are by far the most common HPA specificities induced by transfusion [2, 3] and also the second and third most frequent alloantibodies causing FNAIT [1]. In contrast, anti-D is the most frequent red cell antibody formed after both pregnancy and transfusion. Therefore, it would appear that the anti-HPA-1a response is unique in that pregnancy is almost essential for its induction.

In fact, pregnancy appears to be a physiological condition that is conducive to alloantibody responses. Most red cell blood groups, and platelet and neutrophil alloantigens, were discovered by serological investigations of fetal alloimmune cytopenias resulting from immunization of pregnant or post-partum women by alloantigens on fetal blood. Anti-D (IgM) was the first to be recognized 70 years ago [4].

The human leucocyte antigen (HLA) system was also elucidated mainly by the use of sera from multigravidae, after studies demonstrating leucocyte antibodies were often induced by pregnancy [5, 6]. These sera were the mainstay of HLA-typing reagents before the molecular era. HLA antibodies are not, however, usually deleterious during pregnancy, and curiously, HLA allorecognition by pregnant women of their semi-allogeneic offspring actually appears

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to be important for successful pregnancy [7]. Fertility is reduced among consanguineous couples, spontaneous abortions being more likely with HLA concordance of mother and fetus [8]. Vertical transmission (mother to fetus) of HIV was found greater (31%) with maximum HLA matching of mother and fetus compared with the protection afforded by no HLA class I match, with only 3% transmission [9]. HLA-C-incompatible pregnancies were associated with induction of T-regulatory lymphocytes (Treg) in maternal decidua [10]. Thus, rather surprisingly, maternofetal mismatch of HLA class I enhances placental function and is beneficial to both mother and baby.

Sir Peter Medawar studied transplantation for which he received the Nobel Prize for Medicine in 1960. He proposed the concept of the fetal allograft and hypothesized that survival of the semi-allogeneic fetus occurs either through anatomical separation of mother and fetus, lack of fetal antigen expression or its exposure to the maternal immune system, or from a functional suppression of maternal lymphocytes [11]. However, pregnant women are not immunosuppressed and readily make alloantibodies to blood cells. The 'Immunological Paradox of Pregnancy' is not yet completely understood.

The placenta and amniotic sac form a physical barrier between the mother and fetus. Knowledge of placental anatomy and recent information from immunological studies at various interfaces may help explain the paradox.

Anatomy and development of the placenta

After conception, cellular division of the fertilized egg forms the blastocyst. This is a ball of cells with the entire outer surface comprised of trophoblast cells and with a cluster of cells in the centre, the embryoid body, from which the embryo and then fetus will develop. On contact of the blastocyst with maternal endometrial epithelial cells bordering the decidua (the lining of the uterus), these cytotrophoblast (CT) cells at the contact site fuse to form syncytiotrophoblast (ST; Fig. 1a). The ST acquires an invasive phenotype. Several enzymes are secreted by ST to degrade the extracellular matrix (ECM) and allow invasion of the blastocyst into the maternal tissue (Fig. 1b,c). At this stage, about 1 week after conception, human chorionic gonadotrophin (hCG) is produced by ST to maintain the pregnancy (this hormone is detected by pregnancy tests). Projections of the ST known as villous sprouts erode the decidua basalis and spaces (lacunae) develop in the ST. These gradually fill with interstitial fluid and later with maternal blood (Fig. 1 d). CT near the embryoblast proliferates and moves out inside the villi, forming a cell layer underlying the ST. Both the CT (trophoblast stem cells) and ST (terminally differentiated, non-proliferative syncytium) lack classical HLA class I. As the villi enlarge and branch, they develop a mesoderm stromal core of ECM. Blood vessels from the developing circulation of the embryo/fetus grow through the ECM (Fig. 1e). Blood flows to and from the developing fetus and placenta via the umbilical cord. Fetal and maternal blood does not normally mix.

By 3 weeks post-conception, columns of proliferative CT protrude from the tips of the chorionic villi, becoming EVT. They migrate into the decidua, forming interstitial trophoblasts, and also into the lumen of spiral arteries (Fig. 1e). Here, these endovascular trophoblasts degrade the endothelium rendering the spiral arteries flaccid, ensuring low resistance to uterine arterial blood flow and enabling maximum oxygenation of the placental villi. These differentiated types of invasive EVT do not express HLA class I A or B antigens but, almost uniquely, express HLA-G (Fig. 2a), a non-classical HLA class I molecule [12].

As the embryo develops into a fetus and protrudes into the uterine lumen, after 3–4 weeks, it is surrounded by the amniotic membrane that forms as an inner lining to the chorionic membrane (Fig. 1f). The fetus is protected by amniotic fluid. When the fetus grows, the chorionic membrane outside the site of placental attachment retains some residual trophoblast cells that contact the inner lining of the uterus. The chorion is the major part of the placenta, comprising blood vessels, connective tissue and trophoblasts. This type of placentation is known as haemochorial because maternal blood is in direct contact with the chorionic villi (Fig. 1f). The structure of the placenta at the implantation site remains essentially the same for the remainder of pregnancy, and it grows to become a disk about $20 \times 2–4$ cm at term (40-week gestation).

As pregnancy progresses, the structure of the villi (Fig. 3 a,b) adapts to maximize placental transport to supply the needs of the growing fetus. The villi become extremely numerous, highly vascular and increasingly slender with multiple branches and lobes (Fig. 3a,b). The capillaries become situated predominantly at the periphery of villi, underlying the ST (Fig. 3c). Villous CT cells no longer form a complete cell layer under the ST.

Placental transport

The chorionic villi transfer nutrients and oxygen to the fetal blood and waste products and carbon dioxide back to the maternal circulation. The ST is an absorptive epithelium and has the major role in transport. It is metabolically very active and has numerous vacuoles evident ultrastructurally (Fig. 3c), many of which are involved in placental transfer of substances including amino acids, low density lipoprotein and IgG. Microvilli (a brush border) on the ST (Fig. 3c) increase the villous surface area tenfold to approximately 100 m^2 to maximize absorption [13].
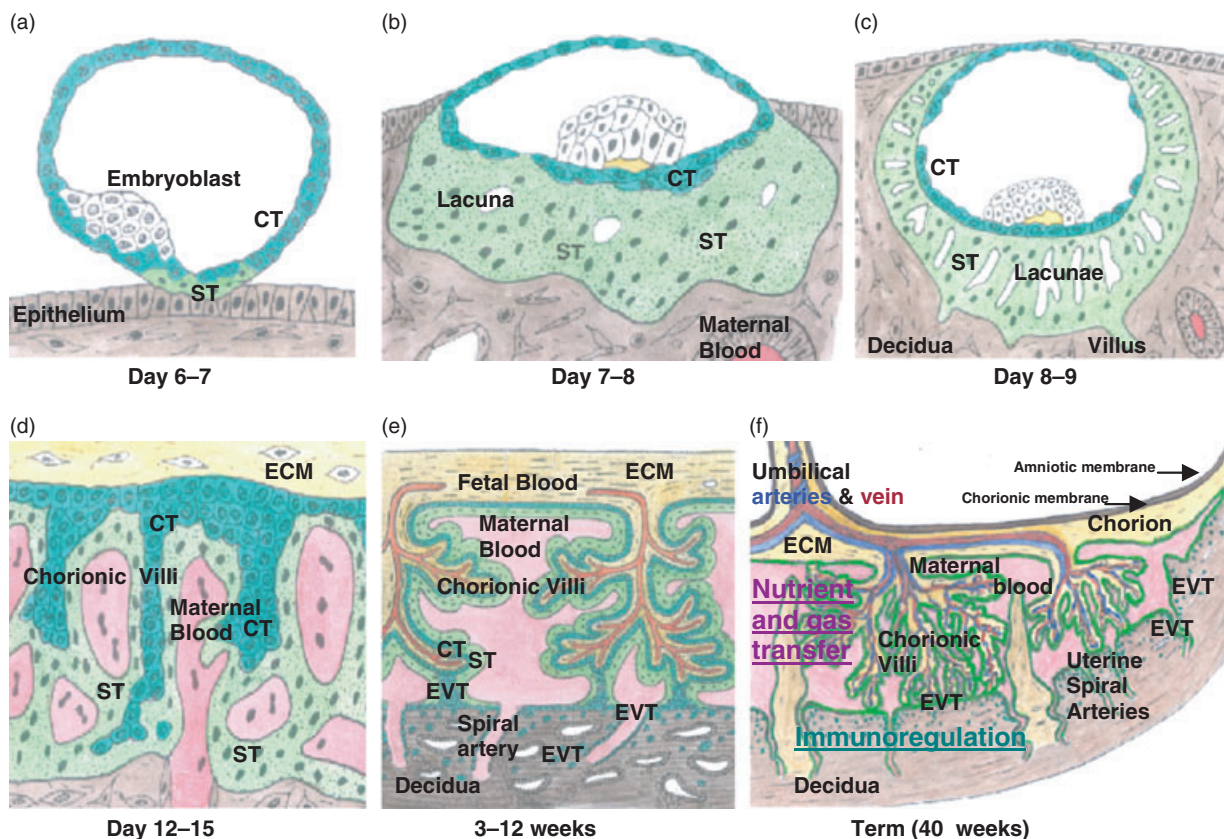
Fig. 1 Development of the placenta. (a) The blastocyst contacts uterine epithelium, and then, syncytiotrophoblast (ST) forms by fusion of cytotrophoblast (CT) cells at this site. (b) As the blastocyst embeds into the decidua, ST proliferates and spaces (lacunae) form in the syncytium. (c) Sprouts (villi) of the syncytium invade the decidua. (d) CT cells by the extracellular matrix (ECM) proliferate and migrate down the villi, whilst the lacunae begin to fill with maternal blood from the uterine arteries and veins. Maternal blood contacts the ST. (e) After about a month when the fetal blood and circulatory system forms, blood vessels in the ECM penetrate the villi and begin to take up nourishment and oxygen from the blood in the intervillous spaces. The villi proliferate and branch. They are covered by a double layer of trophoblast, CT cells and ST. At the tips of villi, CT cells proliferate to form cell columns that migrate into the interstitial tissue in the decidua and into the spiral arteries where they replace the endothelium. These are all extravillous trophoblasts. (f) The CT cells and ST not at the contact site mostly degenerate but some remain on the chorionic membrane, which is attached to the amniotic membrane that contains amniotic fluid surrounding the fetus. Placental transfer takes place in the chorionic villi and immunoregulation in the decidua.

Materno-fetal transport of IgG takes place to confer passive immunity to the fetus, so that after birth, the infant will be protected against infections whilst its own immune system is developing. Active transport of IgG occurs via the neonatal Fc receptor (FcRn) in the ST [14, 15]. Maternal plasma is endocytosed at the apical membrane of the ST, and intracellularly, the endocytotic vacuoles become slightly acidified to approximately pH 6·0 compared with pH 7·4 of plasma. FcRn is present on the ST and endosomal membranes, and then under the acidified conditions, it binds IgG through histidine residues on the CH2 and CH3 domains of IgG. Sorting of the endosomes then occurs, with vacuoles containing IgG–FcRn complexes being transferred across the cell, whilst the contents of those vacuoles lacking these complexes undergo lysosomal degradation [16]. IgM and IgA are not transferred because they have no specific receptors. On reaching the basal (fetal) side of the ST, the endosomes merge with the plasma membrane, and with the rise in pH of the interstitial fluid, IgG dissociates and is released intact, from where it diffuses into the fetal capillaries.

## Fetal material released into maternal blood

Three types of fetal or placental material can be detected in the maternal circulation (Fig. 3c) [17]. Fetal blood may enter, by fetomaternal haemorrhage (FMH), after damage to chorionic villi. In contrast, fetal (placental) DNA and ST debris are detectable in maternal blood from about 7 weeks of gestation, in increasing amounts as the placenta grows.

Over half a century ago, it was recognized that fetal red-blood-cells (RBC) could leak into maternal blood through transplacental haemorrhage and, if fetal RBC were D-positive and the mother was D-negative, could stimulate her to
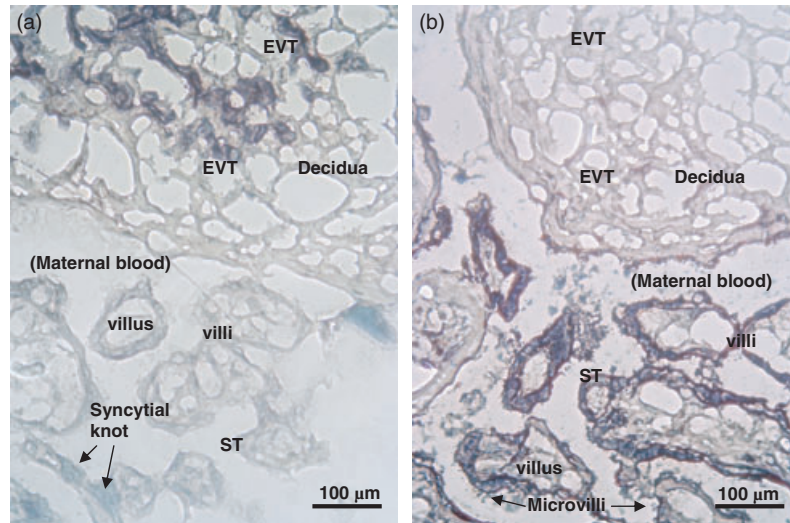
Fig. 2 Immunocytochemistry of decidua and placental chorionic villi. Cryostat sections were stained with (a) anti-HLA-G (purple) and (b) anti-(placental alkaline phosphatase) PLAP (purple). The nuclei are counterstained with methyl green. (a) Extravillous trophoblast (EVT) cells in the decidua are HLA-G-positive, whilst syncytiotrophoblast (ST) in the chorionic villi is not stained. A syncytial knot (green) is between two villi. (b) On chorionic villi, ST membranes and microvilli are PLAP positive, as is particulate material in the intervillous space where maternal blood circulates.
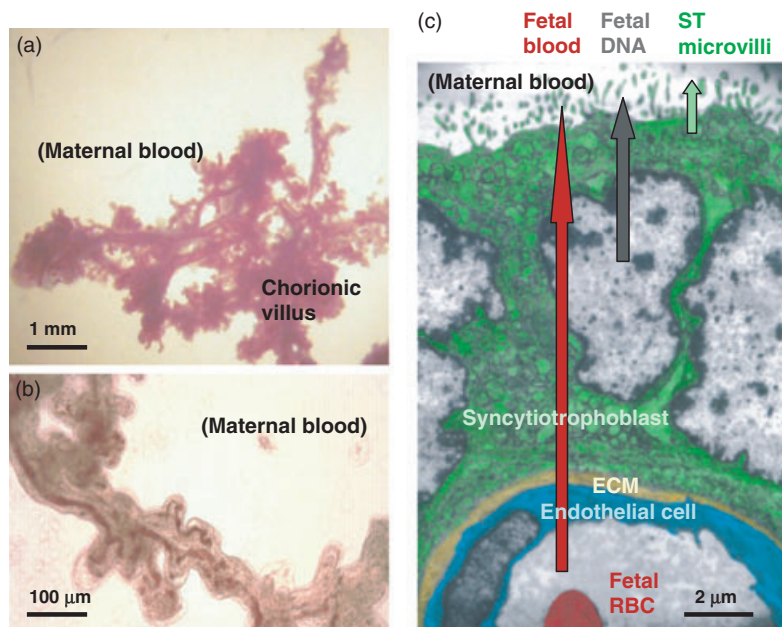
Fig. 3 (a,b) Low power photomicrographs of a portion of a villus, dissected from a term placenta, washed free of maternal blood. Fetal blood vessels and bulbous protuberances are evident in (b). (c) Electron micrograph of the outer portion of a villus, showing syncytiotrophoblast (ST) with nuclei and microvilli, extracellular matrix and a fetal capillary. Three types of fetoplacental material are released into maternal blood: fetal blood (only if the integrity of the villus is lost), apoptotic ST nuclei and DNA, and ST microvillous membrane particles.

produce anti-D, causing HDFN [18]. The ultrastructure of chorionic villi shows that fetal blood must pass through breaks in the capillary endothelial cells, ECM and ST (Fig. 3 c) [17]. FMH are usually unpredictable [19]. Because the

fetoplacental blood volume is approximately 25, 150 and 400 ml at 20-, 30- and 40-weeks gestation, respectively, the occurrence and volume of FMH increases as pregnancy progresses. FMH are more likely at parturition and often in

larger bleeds [20, 21]. Only a minority of susceptible D-negative women with D-positive babies make anti-D, because most are not exposed to enough fetal RBC. Approximately 0·1 ml or less was required to stimulate anti-D formation [22]. The low volume of fetal RBC entering maternal blood during pregnancy also accounts for the fact that the formation of anti-D is rare (<1%) during first pregnancies [23]. The fact that the incidence of anti-HPA-1a alloimmunization in first pregnancies of HPA-1a negative women was found higher (4% [24], 7·6% [25], 24% [26]) when the number of fetal platelets encountered by the maternal immune system from FMH must be very low, suggests this antigen is highly immunogenic during pregnancy.

The ST is a dynamic cellular tissue that is continually remodelled. The nuclei of ST survive for approximately 3 weeks [27] when they then become apoptotic. This is characterized by the appearance of pyknotic (odd shaped) nuclei, condensation of chromatin at the periphery of the nucleus [17], degradation of the nuclear membrane and nuclear shrinkage [28]. DNA fragmentation occurs; endonucleases cleave DNA at internucleosomal linker regions into short (∼200 bp) fragments [29]. The apoptotic nuclei collect into clusters in syncytial knots [30] that are then extruded into maternal blood for disposal (Fig. 2a). Fibrinoid material laid down from maternal blood covers damaged areas of villi [31]. The syncytial layer is rapidly replaced by fusion of underlying CT with ST to maintain the integrity of the villous tissue and prevent haemorrhages. The DNA released from these apoptotic placental ST nuclei is the source of cell-free 'fetal' DNA used for non-invasive fetal genotyping [32] of *RHD* [33], *HPA-1A* [34, 35], *SRY* (gender) and other fetal genes.

Shedding of ST debris in the form of microparticulate material is also a physiological process. Using antibodies to a placental-specific molecule highly expressed on the ST apical membrane, placental alkaline phosphatase (PLAP) [36], it can be seen that this membrane appears 'rough' because of staining of the microvilli (Fig. 2b). An ultrahistochemical study localized PLAP to the ST membrane and microvilli [37]. PLAP enzyme activity in maternal plasma rises during the third trimester [38]. PLAP-positive microparticles are present in the intervillous spaces where maternal blood circulates (Fig. 2b). These ST membrane fragments, vesicles or microparticles, here called syncytiotrophoblast microparticles (STMP), are <500 nm [39] and may have an important role in maternal systemic immunity.

Functions of trophoblast

Trophoblast cells form the fetomaternal interface. EVT cells are in contact with maternal tissue and cells in the decidua.

ST on the surface of the villi is bathed in maternal blood. All trophoblast cells lack classical HLA class I A and B antigens, suggesting a role in immune evasion, but their physiological and immunological functions are quite dissimilar at these two interfaces.

Immunoregulation by placental cells in the decidua

Several mechanisms involving different cells and molecules in decidual tissue are involved in maintaining placental function and survival of the fetoplacental unit. First, maternal natural killer (NK) cells, CD8+ cytotoxic T cells, macrophages and dendritic cells (DC) are affected by ligation with HLA class I expressed on EVT, both HLA-C (classical) and, importantly, HLA-G (non-classical). Second, the location and function of maternal NK cells, Tregs and DCs are altered by placental hormones. Most interactions downregulate immune responses and promote tolerance by preventing killing of allogeneic cells. In the first trimester, this is essential for placental growth and tissue remodelling.

Uterine NK (uNK) cells have a slightly different phenotype to those in peripheral blood, being CD16^{dim} and CD56^{bright} and they reside in the decidua at high concentration early in pregnancy, their proliferation stimulated by hCG [40]. uNK cells express killer inhibitory receptors (KIRs) specific for HLA class I on EVT. HLA-C on EVT interacts with KIR2DL1,2,3 and KIR2DS1,2 (the sole activating receptor) on uNK. There are two groups of HLA-C alleles: C1 binds KIR2DL2,3 and C2 binds KIR2DL1 [41]. Some KIR/HLA-C combinations lead to ineffective invasion of EVT, associated with the disease of pre-eclampsia [42]. However, in HPA-1a alloimmunized pregnancies, no KIR/HLA-Cw gene combinations were found to have a detrimental or protective effect [43].

Uterine NK cells are also downregulated by interaction with the non-classical HLA class I molecules expressed on EVT. The most important is HLA-G that inhibits uNK through KIR2DL4, NKG2A (CD94), p49 and immunoglobulin-like transcript (ILT) receptor ILT2 (CD85j) on uNK cells [41, 44]. HLA-E is present at low concentration on EVT and binds NKG2A on uNK cells [41].

CD8+ effector memory T cells in the decidua are rendered non-functional because production of cytotoxic molecules is downregulated, thus sparing EVT from cytolysis [45]. Experimentally, activated CD8+ lymphocytes were triggered into apoptosis by soluble rHLA-G1, mediated through the CD95/CD95 ligand (Fas/Fas ligand) pathway, suggesting that this mechanism may explain the paucity of CD8+ cells in the decidua [46].

Maternal macrophages are present in the decidua throughout pregnancy and secrete immunoregulatory molecules, interleukin (IL)-10 [47], IL-1Ra and indoleamine 2,3

dioxygenase (IDO) [48]. (IDO catabolizes tryptophan and suppresses T cells by reducing the concentration of this essential amino acid required for their metabolism.) Decidual macrophages express the inhibitory receptors ILT2 and ILT4 that bind HLA-G dimers, recently identified on the surface of first-trimester EVT [49].

Some DCs reside in the decidua but are rendered immature or tolerant by progesterone (produced by the placenta) and experimentally by interacting with HLA-G through ILT2 (LILRB1) [50]. The DCs secrete immunoregulatory cytokines; IL-10 promotes Tregs, and IL-4, IL-10 and TGFβ drive T-cell differentiation down the T-helper (Th)2/3 pathway, away from cytotoxic T cells [51].

Finally, Tregs (CD4$^+$, CD25^{++}) accumulate in the decidua despite decreasing in the maternal circulation during pregnancy [52, 53], attracted by hCG [54]. Their concentration in the decidua may reach 14% of the T cells, much higher than in peripheral blood [55]. Lack of Tregs leads to rejection of the fetus [56].

Maternal cells in the decidua are not completely suppressed, however. Macrophages actively recognize and phagocytose pathogens, which is important in protection against intrauterine infections [57].

Systemic immunomodulation

A state of mild systemic activation of the innate immune system exists during pregnancy, especially in the third trimester, with increased numbers of monocytes and granulocytes found in maternal blood [58]. Monocytes from pregnant women had enhanced production of IL-12 *in vitro* compared with monocytes from non-pregnant women [59]. STMP shed into maternal blood may be responsible for this. *In vitro*, STMP were found to stimulate the release by monocytes of the inflammatory cytokines IL-1β, IL-6 and IL-8 [60] and TNF-α, IL-12p70 and IL-18 [61] and by DC of IL-6 and IL-8 [62]. Innate immunity and inflammation are necessary for priming antigen-presenting cells (macrophages, DC) to initiate T-helper (Th) and B-cell immune responses (Fig. 4).

Villous trophoblasts may also affect the systemic immune system via production of cytokines. Isolated villous trophoblast cells (ST and CT) in culture were found to secrete IL-10 [63–65], to express mRNA for IL-10, IL-4 and IL-13 [65] and to produce IL-10 receptor mRNA and protein [66] suggesting a strong Th2 bias of these cells. Progesterone and hCG, hormones synthesized by ST, caused shifts of immunity from Th1 (IL-2, IL-12, IL-15, interferon-γ; cell-mediated) to Th2 (IL-4, IL-5, IL-6, IL-10, IL-13; humoral) [67]. NK cells were reduced in the blood of pregnant women [58]. The number of Tregs in peripheral blood of pregnant women was markedly elevated during early pregnancy, then after mid-gestation returned to normal levels at term

[68], indicating different mechanisms operate at the beginning of pregnancy, when the fetal–placental unit is being established, and later, when fetal growth is of major importance.

Therefore, a complex mix of systemic immune activation and a cytokine bias towards Th2, first proposed by Wegman in 1993 [69], appears to be induced in pregnant women. The low-level inflammatory state combined with skewing towards humoral immunity may explain why antibody responses towards small volumes of allogeneic fetal blood cells are robust. Th1 cell-mediated immunity is, however, somewhat compromised, evidenced by increased susceptibility of pregnant women to viral (e.g. swine flu, H1N1) and intracellular bacterial (e.g. listeria monocytogenes) infections, with the latter carrying a high risk of fetal loss if the placenta is colonized.

Could STMP stimulate the maternal anti–HPA–1a immune response?

The glycoprotein (GP) that carries the HPA-1 polymorphism (GPIIIa, CD61 or β3 integrin) was found biochemically to be expressed on the ST 'brush border' (i.e. STMP) prepared from term placentas [70]. It was later shown to be present on first-trimester ST as well as term ST by electron microscopy [17]. On ST, GPIIIa is associated with CD51 (αV integrin) and not with GPIIb (CD41, αIIb integrin) as the latter is expressed on platelets but was not detected on ST by immunocytochemistry [71]. Binding of anti-HPA-1a to these two complexes may differ.

STMP in maternal blood are likely to gain access to lymph nodes and the spleen, where they could be endocytosed by macrophages or DC. This endocytosis is non-specific; DCs are continually sampling their environment. After antigen processing, peptides are presented on HLA class II on the DC surface for presentation to CD4$^+$ Th cells. In FNAIT, there is a very strong HLA DRB3*0101 (DRw52a) restriction of the HPA-1a antibody response [72]. Therefore, peptides encompassing the polymorphic residues from GPIIIa will be bound to DRB3*0101 molecules [73–75]. On the DC surface, this HPA-1a-peptide complexed to HLA would select HPA-1a-specific Th (CD4$^+$) cells (Fig. 4). These cells must have T-cell receptors (TCR) that recognize a complex of HPA-1a peptide bound to DRB3*0101, the restricting HLA molecule. If the HPA-1a-specific Th cells receive stimulation from the DC (cytokines and interaction with membrane costimulatory receptors), the Th cells become activated, proliferate and migrate to B-cell areas. HPA-1a-specific T cells were identified in HPA-1a-immunized women by culturing peripheral blood mononuclear cells with HPA-1a peptides and measuring T-cell proliferation; most of the pregnant and post-partum women studied were found to have HPA-1a-specific T cells, whereas
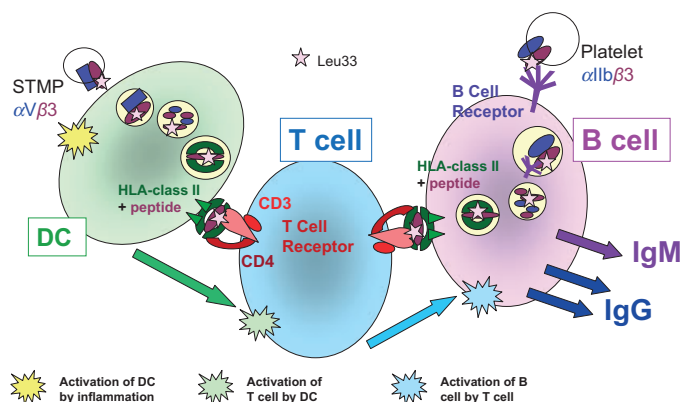
Fig. 4 The antibody response (summarized). Dendritic cells (DC) endocytose plasma and particulate matter non-specifically; the contents of the endocytotic vacuoles are digested; peptides are bound to HLA class II molecules and presented on the cell surface for recognition by T-cell receptors (TCR) on CD4^{+} T-helper (Th) lymphocytes. If the conformation of the TCR enables it to bind the peptide–HLA complex with high affinity and the Th cell receives stimulation from the DC in the form of cytokines and cell-surface costimulatory molecules, the Th cell becomes activated. It then proliferates, and the clonal progeny migrates to B cell-rich areas of lymph nodes and spleen. B cells recognize their cognate antigen through conformational binding of the B-cell receptor (BCR; surface IgM) to the antigen. This stimulates endocytosis and then antigen processing and presentation in a similar way to that of the DC. If the B cells are contacted by Th cells with the correct TCR to bind the peptide–HLA complex, the B cell will be activated by the Th cell, proliferate and secrete antibody (IgM, IgG) of the same specificity as the BCR.

non-HPA-1a-immunized individuals did not [76]. These T-cell responses were generally stronger during pregnancy [76] than from non-pregnant women [77]. This illustrates that exposure to HPA-1a during pregnancy stimulates specific immune responses in susceptible women.

Antibody response

B-lymphocyte responses are initiated by recognition of the conformational shape of antigens by B-cell receptors (BCRs). The BCR (surface IgM) binds antigen that mediates endocytosis of the BCR–antigen complex followed by antigen processing and presentation of peptides on HLA class II for recognition by antigen-specific Th cells. Thus, to receive help (activation) from Th cells, the B cells must present the same peptide–HLA class II complex as the DC that initially activated the Th cells (Fig. 4). Following help in the form of cytokines and costimulatory molecules from these Th cells, the antigen-specific B cells become activated, proliferate and produce antibodies of the same specificity as their BCR. Therefore, to produce an antibody that binds to HPA-1a on platelets, the maternal B cells must have endocytosed HPA-1a-positive platelets. These can only be encountered by the maternal immune system by FMH. As mentioned earlier, FMH are generally unpredictable, infrequent and small, probably containing usually fewer than 25×10^6 fetal platelets – a very small number to cause a primary immune response in an adult. However, if antigen-specific Th cells are already present in high numbers and ready to activate the antigen-specific B cells, this low dose may be sufficient.

Pregnant women appear to make alloantibodies more readily than transfused patients, considering the great difference in volumes of blood in FMH (rarely over 20 ml) compared with transfusions. Interestingly, without prophylaxis, approximately 10–20% of susceptible (antigen mismatched) gravid women develop alloantibodies, 16% with anti-D [78], 12% and 10% with anti-HPA-1a [26, 79] and 15% with anti-HLA [80], indicating a similar immunization by FMH.

Maternal alloantibodies

Immunizations

The initial antibody response to an antigen is predominantly IgM, of high avidity and agglutinating activity but relatively low specificity and affinity. Re-immunization with antigen is required to mature the antibody response. (This is the purpose of booster injections after vaccination.) Thus, in order that high-titre maternal alloantibodies to fetal blood alloantigens may develop, further doses of antigen (i.e. via FMH) are required.

Class switching and affinity maturation

With further antigen encounter, the B cells switch antibody class from IgM to IgG by selection of Cγ genes for IgG, to replace Cμ (IgM) genes. The Cγ3 gene (coding for IgG3) is upstream of Cγ1 (coding for IgG1); therefore, IgG3 may convert to IgG1 but not the reverse. By a process of somatic

mutation (point mutations of the variable regions of Ig), affinity for antigen increases. The cytokine environment of the B cells affects the class and subclass of antibody. Because additional FMH may or may not occur, maturation of the antibody response may be halted during this process (Fig. 5), resulting in heterogeneity of alloantibody isotype, titre and affinity among different antisera [81].

Isotype and functional activity

Most alloantibodies to red cells and platelets are IgG1, sometimes IgG3 or a mixture [82, 83]. IgG is the immunoglobulin class that has most effector functions, mediating adherence, phagocytosis and lysis of IgG-coated cells by IgG Fc receptors (FcγR) on macrophages. Several FcγR are present on macrophages and granulocytes (neutrophils, eosinophils, basophils, mast cells, NK cells) [84]. Complement fixation on the opsonized target cell may also occur. IgG enhances and accelerates the innate immune mechanisms of destruction of target cells by these effector cells. IgG is the only antibody class to cross the placenta to the fetus.

Glycosylation

The glycosylation of IgG also modulates its effector function [85]. The branched core oligosaccharide structure attached to Asn297 on the CH2 domain of IgG has several sugars that may or may not be present [86] (Fig. 6). Terminal galactose is elevated on IgG of pregnant women [87] and is even higher on umbilical cord (fetal) IgG [88]. Galactose on IgG enhances effector cell activity by macrophages [89]. In contrast, fucose reduces effector activity [90]. Affinity-purified IgG1 anti-HPA alloantibodies were found
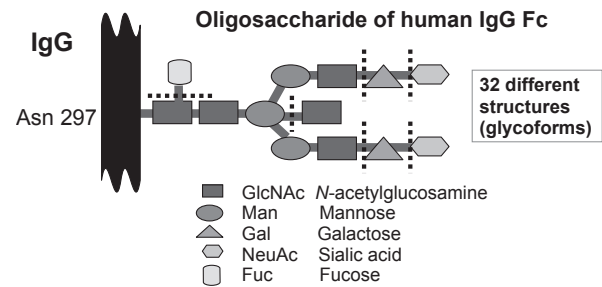
Fig. 6 Diagram of the composition of sugars of the oligosaccharide chain attached to Asparagine 297 in the CH2 (constant) domains of IgG. The core structure terminates in GlcNAc, at the dotted lines, and complexity arises because additional sugars (galactose, sialic acid, fucose and bisecting sialic acid) may or may not be present.

to have less fucose and slightly higher galactose, compared with normal serum IgG1 from the donors, thus enhancing their pathogenicity [91]. This physiological elevation of antibody function may have evolved to help protect pregnant women from pathogens whilst their immune systems are in a state of mild systemic immunoregulation.

Concluding remarks

Human pregnancy is unique in that it allows the growth of a fetus across highly polymorphic HLA mismatches whilst the maternal and fetal immune systems are not compromised. The structure of the placenta and its effect on maternal immune cells drives this change. Skewing of maternal systemic immunity towards humoral responses ensures development of protective antibodies. Materno-fetal–placental transfer of IgG confers passive immunity to the infant. Formation of alloantibodies to alloantigens on fetal
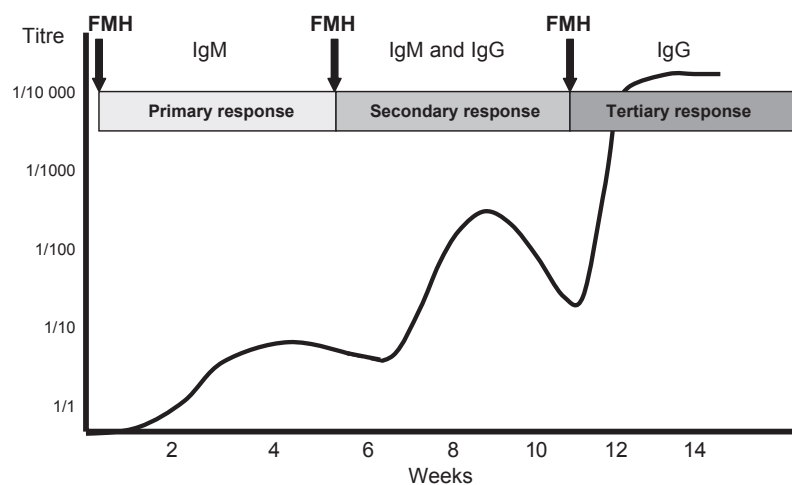
Fig. 5 Antibody development. The diagram represents the titre and Ig class of an alloantibody formed in response to one or more immunizations in the form of fetomaternal haemorrhage (FMHs). If there are no secondary or tertiary FMH, the maternal antibody will remain of low titre (weak) and predominantly IgM. (The scale is arbitrary.)

blood cells acquired by FMH which then result in alloimmune cytopenias is, unfortunately, a consequence of physiological rather than pathological processes.

References

1 Davoren A, Curtis BP, Aster RH, *et al.*: Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. *Transfusion* 2004; **44**:1220–1225

2 Kiefel V, König C, Kroll H, *et al.*: Platelet *et al.* loantibodies in transfused platelets. *Transfusion* 2001; **41**:766–770

3 Engelfriet CP, Reesink HW: Detection of platelet-reactive antibodies in patients who are refractory to platelet transfusions, and the selection of compatible donors. *Vox Sang* 2003; **84**:73–88

4 Levine P, Katzin EM, Burnham L: Isoimmunization in pregnancy: its possible bearing on the etiology of erythroblastosis fetalis. *JAMA* 1941; **116**:825–827

5 Van Rood JJ, Eernisse JG, van Leeuwen A: Leococyte antibodies in sera from pregnant women. *Nature* 1958; **181**:1735–1736

6 Terasaki P, McClelland JD: Microdroplet assay of human serum cytotoxins. *Nature* 1964; **204**:998–1000

7 Rugeles MT, Shearer GM: Alloantigen recognition *in utero*: dual advantage for the fetus? *Trends Immunol* 2004; **25**:348–352

8 Ober C, Hyslop T, Elias S, *et al.*: Human leukocyte antigen matching and fetal loss: results of a 10 year prospective study. *Hum Reprod* 1998; **13**:33–38

9 MacDonald KS, Embree J, Njenga S, *et al.*: Mother-child class I HLA concordance increases perinatal human immunodeficiency virus type 1 transmission. *J Infect Dis* 1998; **177**:551–556

10 Tilburgs T, Scherjon SA, van der Mast BJ, *et al.*: Fetal-maternal HLA-C mismatch is associated with decidual T cell activation and induction of functional T regulatory cells. *J Reprod Immunol* 2009; **82**:148–157

11 Medawar PD: Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp Soc Exp Biol* 1953; **7**:320–338

12 Ellis SA, Sargent IL, Redman CW, *et al.*: Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line. *Immunology* 1986; **59**:596–601

13 Teasdale F, Jean-Jacques G: Morphometric evaluation of the microvillous surface enlargement factor in the human placenta from mid-gestation to term. *Placenta* 1985; **6**:375–381

14 Simister NE, Story CM, Chen HL, *et al.*: An IgG-transporting Fc receptor expressed in the syncytiotrophoblast of human placenta. *Eur J Immunol* 1996; **26**:1527–1531

15 Leach JL, Sedmak DD, Osborne JM, *et al.*: Isolation from human placenta of the IgG transporter, FcRn, and localization to the syncytiotrophoblast: implications for maternal-fetal antibody transport. *J Immunol* 1996; **157**:3317–3322

16 Ghetie V, Ward ES: FcRn: the MHC class I-related receptor that is more than an IgG transporter. *Immunol Today* 1997; **18**:592–598

17 Kumpel BM, Sibley K, Jackson DJ, *et al.*: Ultrastructural localization of glycoprotein IIIa (GPIIIa, beta 3 integrin) on placental syncytiotrophoblast microvilli: implications for platelet *et al.* loimmunization during pregnancy. *Transfusion* 2008; **48**:2077–2086

18 Chown B: Anemia from bleeding of the fetus into the mother's circulation. *Lancet* 1954; **1**:1213–1215

19 Sebring ES, Polesky HF: Fetomaternal hemorrhage: incidence, risk factors, time of occurrence, and clinical effects. *Transfusion* 1990; **30**:344–357

20 Woodrow JC, Finn R: Transplacental haemorrhage. *Br J Haematol* 1966; **12**:297–309

21 Bowman JM, Pollock JM, Penston LE: Fetomaternal transplacental hemorrhage during pregnancy and after delivery. *Vox Sang* 1986; **51**:117–121

22 Zipursky A, Israels LG: The pathogenesis and prevention of Rh immunization. *Can Med Assoc J* 1967; **97**:1245–1257

23 Urbaniak S: The clinical application of anti-D prophylaxis; in Hadley A, Soothill P (eds): Alloimmune Disorders of Pregnancy. Cambridge, Cambridge University Press, 2002: 97–119

24 Turner ML, Bessos H, Fagge T, *et al.*: Prospective epidemiologic study of the outcome and cost-effectiveness of antenatal screening to detect neonatal alloimmune thrombocytopenia due to anti-HPA-1a. *Transfusion* 2005; **45**:1945–1956

25 Killie MK, Husebekk A, Kjeldsen-Kragh J, *et al.*: A prospective study of maternal anti-HPA-1a antibody level as a potential predictor of alloimmune thrombocytopenia in the newborn. *Haematologica* 2008; **93**:870–877

26 Williamson LM, Hackett G, Rennie J, *et al.*: The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PlA1, Zwa) as determined by antenatal screening. *Blood* 1998; **92**:2280–2287

27 Huppertz B, Frank HG, Kingdom JC, *et al.*: Villous cytotrophoblast regulation of the syncytial apoptotic cascade in the human placenta. *Histochem Cell Biol* 1988; **110**:495–508

28 Kerr JF, Wyllie AH, Currie AR: Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. *Br J Cancer* 1972; **26**:239–257

29 Holdenrieder S, Stieber P, Bodenmüller H, *et al.*: Circulating nucleosomes in serum. *Ann N Y Acad Sci* 2001; **945**:93–102

30 Jones CJ, Fox H: Syncytial knots and intervillous bridges in the human placenta: an ultrastructural study. *J Anat* 1977; **124**:275–286

31 Nelson DM, Crouch EC, Curran EM, *et al.*: Trophoblast interaction with fibrin matrix. Epithelialization of perivillous deposits as a mechanism for villous repair in the human placenta. *Am J Pathol* 1990; **136**:855–865

32 Lo YM, Corbetta N, Chamberlain PF, *et al.*: Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997; **350**:485–487

33 Finning KM, Martin PG, Soothill PW, *et al.*: Prediction of fetal D status from maternal plasma: introduction of a new noninvasive fetal *RHD* genotyping service. *Transfusion* 2002; **42**:1079–1085

34 Scheffer PG, de Haas M, van der Schoot CE: Non-invasive fetal blood typing for

platelet and red cell antigens. *Vox Sang* 2010; **99**(Suppl 2):11

35 Freixa L, Nogues N, Ibanez M, *et al.*: Development and validation of non-invasive approach for fetal HPA-1a genotyping using cell-free fetal DNA present in maternal plasma. *Vox Sang* 2010; **99**(Suppl 2):22

36 Manoussaka MS, Jackson DJ, Lock RJ, *et al.*: Flow cytometric characterisation of cells of differing densities isolated from human term placentae and enrichment of villous trophoblast cells. *Placenta* 2005; **26**:308–318

37 Jones CJ, Fox H: An ultrahistochemical study of the distribution of acid and alkaline phosphatases in placentae from normal and complicated pregnancies. *J Pathol* 1976; **118**:143–151

38 Hunter RJ, Pinkerton JH, Johnston H: Serum placental alkaline phosphatase in normal pregnancy and preeclampsia. *Obstet Gynecol* 1970; **36**:536–546

39 Smith CH, Nelson DM, King BF, *et al.*: Characterization of a microvillous membrane preparation from human placental syncytiotrophoblast: a morphologic, biochemical, and physiologic study. *Am J Obstet Gynecol* 1977; **128**:190–196

40 Kane N, Kelly R, Saunders PT, *et al.*: Proliferation of uterine natural killer cells is induced by human chorionic gonadotropin and mediated by the mannose receptor. *Endocrinology* 2009; **150**:2882–2888

41 Moffet-King A: Natural killer cells and pregnancy. *Nat Rev Immunol* 2002; **2**:656–663

42 Moffet A, Hiby SE: How does the maternal immune system contribute to the development of pre-eclampsia? *Placenta* 2007; **28**(Suppl 1):S51–S56

43 Valentin N, Gagne K, Kerdudou N, *et al.*: The alloimmune response to the human platelet antigen-1a is not related to maternal-fetal killer immunoglobulinlike receptor/HLA-Cw combinations. *Transfusion* 2007; **47**:2322–2329

44 Ponte M, Cantoni C, Biassoni R, *et al.*: Inhibitory receptors sensing HLA-G1 molecules in pregnancy: decidua associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, and HLA-G1-specific receptor. *Proc Natl Acad Sci USA* 1999; **96**:5674–5679

45 Tilburgs T, Schonkeren D, Eikmans M, *et al.*: Human decidual tissue contains differentiated CD8+ effector-memory T cells with unique properties. *J Immunol* 2010; **185**:4470–4477

46 Fournel S, Aguerre-Girr M, Huc X, *et al.*: Cutting edge: soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8+ cells by interacting with CD8. *J Immunol* 2000; **164**:6100–6104

47 McIntyre RH, Ganacias KG, Hunt JS: Programming of human monocytes by the uteroplacental environment. *Reprod Sci* 2008; **15**:437–447

48 Munn DH, Zhou M, Attwood JT, *et al.*: Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 1998; **281**:1191–1193

49 Apps R, Gardner L, Sharkey AM, *et al.*: A homodimeric complex of HLA-G on normal trophoblast cells modulates antigen-presenting cells *via* LILRB1. *Eur J Immunol* 2007; **37**:1924–1937

50 Ristich V, Liang S, Zhang W, *et al.*: Tolerization of dendritic cells by HLA-G. *Eur J Immunol* 2005; **35**:1133–1142

51 Piccinni MP, Giudizi MG, Biagiotti R, *et al.*: Progesterone favours the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 clones. *J Immunol* 1995; **155**:128–133

52 Tilburgs T, Roelen DL, van der Mast BJ, *et al.*: Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. *J Immunol* 2008; **180**:5737–5745

53 Guerin LR, Prins JR, Robertson SA: Regulatory T cells and immune tolerance in pregnancy: a new target for infertility treatment? *Hum Reprod Update* 2009; **15**:517–535

54 Schumacher A, Brachwitz N, Sohr S, *et al.*: Human chorionic gonadotropin attracts regulatory T cells into the fetal-maternal interface during early human pregnancy. *J Immunol* 2009; **182**:5488–5497

55 Heikkinen J, Möttönen M, Alanen A, *et al.*: Phenotypic characterization of regulatory T cells in the human decidua. *Clin Exp Immunol* 2004; **136**:373–378

56 Aluvihare VR, Kallikourdis M, Betz AG: Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004; **5**:266–271

57 Singh U, Nicholson G, Urban BC, *et al.*: Immunological properties of human decidual macrophages – a possible role in intrauterine immunity. *Reproduction* 2005; **129**:631–637

58 Sacks G, Sargent I, Redman C: An innate view of human pregnancy. *Immunol Today* 1999; **20**:114–118

59 Sacks GP, Redman CW, Sargent IL: Monocytes are primed to produce the Th1 type cytokine IL-12 in normal human pregnancy: an intracellular flow cytometric analysis of peripheral blood mononuclear cells. *Clin Exp Immunol* 2003; **131**:490–497

60 Messerli M, May K, Hansson SR, *et al.*: Feto-maternal interactions in pregnancies: placental microparticles activate peripheral blood monocytes. *Placenta* 2010; **31**:106–112

61 Germain SJ, Sacks GP, Sooranna SR, *et al.*: Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles. *J Immunol* 2007; **178**:5949–5956

62 Eastlake J, Kumpel B: Developing in vitro and in vivo model systems to investigate de novo human anti-HPA-1a antibody formation in neonatal alloimmune thrombocytopenia (NAIT). *Vox Sang* 2010; **99**(Suppl 2):19

63 Roth I, Corry DB, Locksley RM, *et al.*: Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10. *J Exp Med* 1996; **184**:539–548

64 Hanna N, Hanna I, Hleb M, *et al.*: Gestational age-dependent expression of IL-10 and its receptor in human placental tissues and isolated trophoblasts. *J Immunol* 2000; **164**:5721–5728

65 Manoussaka MS, Whittle CJ, Kumpel BM, *et al.*: Isolation and characterisation of human term trophoblasts. *Immunology* 1997; **92**(Suppl 1):70

66 Manoussaka MS: Characterisation and cytokine expression of human term placental trophoblasts. PhD thesis, UK, University of Bristol, 2001

67 Piccinni MP, Scaletti C, Maggi E, *et al.*: Role of hormone-controlled Th1- and Th2-cytokines in successful pregnancy. *J Neuroimmunol* 2000; **109**:30–33

68 Zhao JX, Zeng YY, Liu Y: Fetal alloantigen is responsible for the expansion of the CD4+CD25+ regulatory cell pool

during pregnancy. *J Reprod Immunol* 2007; **75**:71–81

69 Wegmann TG, Lin H, Guilbert L, *et al.*: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993; **14**:353–356

70 Vanderpuye OA, Labarrere CA, McIntyre JA: A vitronectin-receptor-related molecule in human placental brush border membranes. *Biochem J* 1991; **280**:9–17

71 Jackson D, Kumpel B: Comparison of integrin expression on human platelets, villous trophoblasts and placental tissue using flow cytometry and immunohistochemistry. *Vox Sang* 2006; **91**(Suppl 2):19

72 Valentin N, Vergracht A, Bignon JD, *et al.*: HLA-DRw52a is involved in alloimmunization against PL-A1 antigen. *Hum Immunol* 1990; **27**:73–79

73 Wu S, Maslanka K, Gorski J: An integrin polymorphism that defines reactivity with alloantibodies generates an anchor for MHC class II peptide binding: a model for unidirectional alloimmune responses. *J Immunol* 1997; **158**:3221–3226

74 Ahlen MT, Husebekk A, Killie MK, *et al.*: T-cell responses associated with neonatal alloimmune thrombocytopenia: isolation of HPA-1a-specific, HLA-DRB3*0101-restricted CD4+ T cells. *Blood* 2009; **113**:3838–3844

75 Rayment R, Kooij TW, Zhang W, *et al.*: Evidence for the specificity for platelet HPA-1a alloepitope and the presenting HLA-DR52a of diverse antigen-specific helper T cell clones from alloimmunized mothers. *J Immunol* 2009; **183**:677–686

76 Jackson DJ, Murphy MF, Soothill PW, *et al.*: Reactivity of T cells from women with antibodies to the human platelet antigen (HPA)-1a to peptides encompassing the HPA-1 polymorphism. *Clin Exp Immunol* 2005; **142**:92–102

77 Sukati H, Bessos H, Barker RN, *et al.*: Characterization of the alloreactive helper T-cell response to the platelet membrane glycoprotein IIIa (integrin-β3) in human platelet antigen-1a alloimmunized human platelet antigen-1b1b women. *Transfusion* 2005; **45**:1165–1177

78 Bowman JM: The prevention of Rh immunization. *Transfus Med Rev* 1988; **2**:129–150

79 Kjeldsen-Kragh J, Killie MK, Tomter G, *et al.*: A screening and intervention program aimed to reduce mortality and serious morbidity associated with severe neonatal alloimmune thrombocytopenia. *Blood* 2007; **110**:833–839

80 Bolckova H, Matejkova E, Zuckove E, *et al.*: HLA allosensibilization of differently immunized persons. *Vox Sang* 2010; **99**(Suppl 2):47

81 Kumpel B, Powell T: Comparison of quantification of anti-D in antenatal sera by flow cytometry and autoanalyser. *Transfus Med* 2007; **17**(Suppl 1):31

82 Mattila PS, Seppala IJ, Eklund J, *et al.*: Quantitation of immunoglobulin classes and subclasses in anti-Rh(D) antibodies. *Vox Sang* 1985; **48**:350–356

83 Proulx C, Filion M, Goldman M, *et al.*: Analysis of immunoglobulin class, IgG subclass and titre of HPA-1a antibodies in alloimmunized mothers giving birth to babies with or without neonatal alloimmune thrombocytopenia. *Br J Haematol* 1994; **87**:813–817

84 Van de Winkel JGJ, Capel PJA: Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol Today* 1993; **14**:215–221

85 Kumpel BM: Efficacy of RhD monoclonal antibodies in clinical trials as replacement therapy for prophylactic anti-D immunoglobulin: more questions than answers. *Vox Sang* 2007; **93**:99–111

86 Takahashi N, Ishii I, Ishihara H, *et al.*: Comparative structural study of the N-linked oligosaccharides of human normal and pathological immunoglobulin G. *Biochemistry* 1987; **26**:1137–1144

87 Pekelharing JM, Hepp E, Kamerling JP, *et al.*: Alterations in carbohydrate composition of serum IgG from patients with rheumatoid arthritis and from pregnant women. *Ann Rheum Dis* 1988; **47**:91–95

88 Williams PJ, Arkwright PD, Rudd P, *et al.*: Short communication: selective placental transport of maternal IgG to the fetus. *Placenta* 1995; **16**:749–756

89 Kumpel BM, Rademacher TW, Rook GA, *et al.*: Galactosylation of human IgG monoclonal anti-D produced by EBV-transformed B-lymphoblastoid cell lines is dependent on culture method and affects Fc receptor-mediated functional activity. *Hum Antibodies Hybridomas* 1994; **5**:143–151

90 Shields RL, Lai J, Keck R, *et al.*: Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. *J Biol Chem* 2002; **277**:26733–26740

91 Wuhrer M, Porcelijn L, Kapur R, *et al.*: Regulated glycosylation patterns of IgG during alloimmune responses against human platelet antigens. *J Proteome Res* 2009; **8**:450–456