

Pathophysiology of the alloimmune cytopenias

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The pathogenesis of the alloimmune cytopenias can be considered in four stages: alloimmunization of the mother, the placental transfer of antibodies to a fetus, the immune destruction of sensitized blood cells and, finally, clinical manifestations which are secondary to the destruction of fetal blood cells such as hydrops, haemorrhage or infection.

1.1 Maternal Alloimmunization

1.1.1 Some key events in the humoral immune response

A comprehensive review of humoral immune responses is outside the scope of this chapter. Nevertheless, a brief consideration of the cells and some of the key processes which result in the production of antibodies is pertinent to several topics covered in this book such as the genetic predisposition to form certain alloantibodies (Section 1.1.3), the mode of action of Rh prophylaxis (Section 5.5), and the basis of new approaches to ameliorate maternal alloimmune responses (Section 14.5). These key steps are shown diagrammatically in Figure 1.1.

Two phases of an immune response are distinguished. The primary response results in very low or undetectable levels of circulating antibody. The second anamnestic response is characterized by much higher concentrations of antibody. The immune response starts with the nonspecific uptake of an antigen by antigen-presenting cells in lymphoid centres such as the spleen and lymph nodes. Internalized antigens are then incorporated into phagolysosomes where they are partially degraded by proteolytic enzymes to peptide fragments. The peptides then associate with HLA class II molecules before being returned to the cell surface and so 'presented' to helper T cells. Interdigitating dendritic cells are particularly efficient at presenting antigen and during a primary immune response they are probably the only cell capable of effective antigen presentation to resting helper T cells. However, B cells can also present antigen and, in secondary immune responses, B

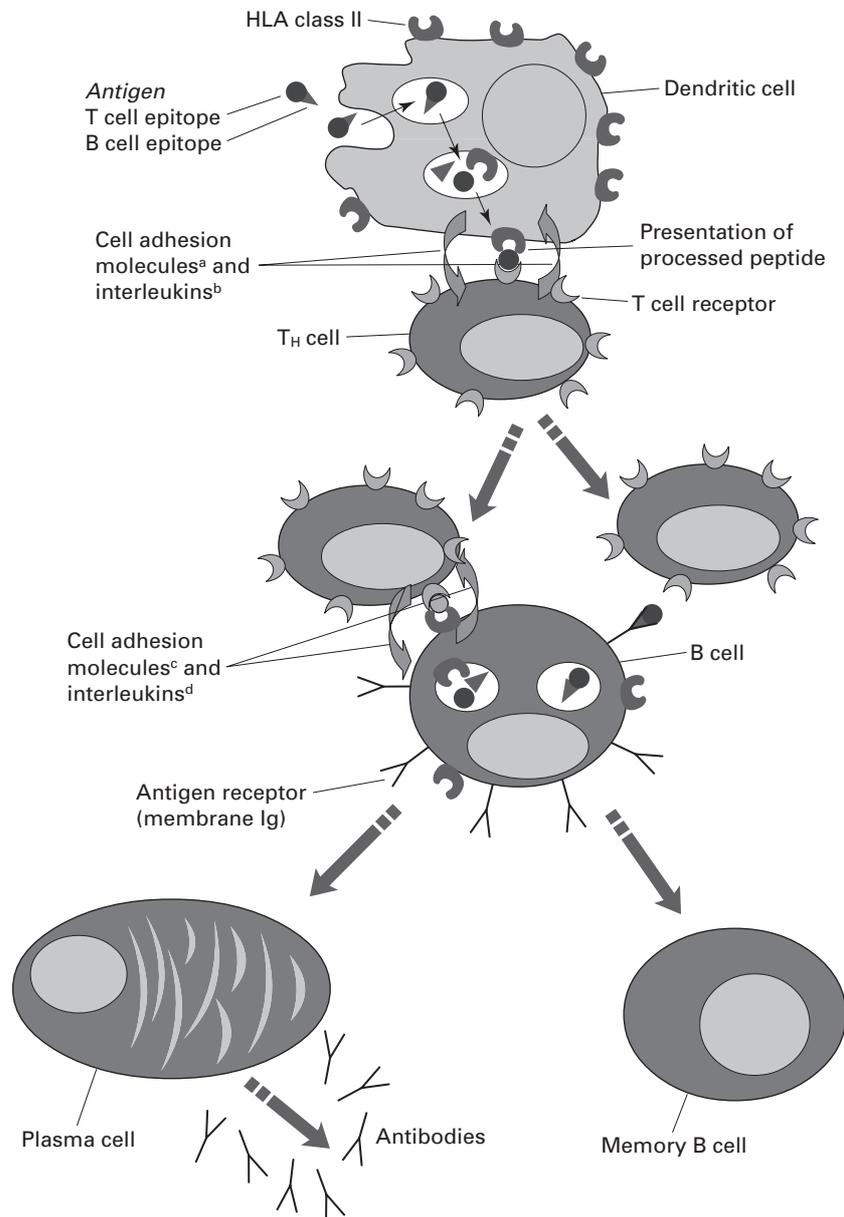


Figure 1.1 Critical steps in the humoral immune response.

Antigens are processed and presented to helper T cells by dendritic cells. In the presence of appropriate secondary signals, helper T cells proliferate and signal antigen-specific B cells to proliferate and secrete antibody.

(a) Co-stimulatory molecules on antigen-presenting cells bind to their ligands on helper T cells; lymphocyte functional antigen 3 (LFA-3) binds to CD2, intercellular adhesion molecule-1 (ICAM-1) binds to LFA-1, CD80/CD86 binds to CD28.

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cells with high affinity immunoglobulin antigen receptors may be the most effective antigen-presenting cells.

As mentioned above, peptides are 'seen' by helper T cells when presented as complexes with HLA class II molecules. The binding of peptides to HLA class II molecules depends on complementarity between the peptide and the groove within the HLA molecule. Since the precise topology of the peptide-binding groove of HLA molecules varies according to the HLA allele(s), the ability to bind peptides derived from certain alloantigens is under genetic control. For example, the ability to form antibodies to the human platelet antigen-1a (HPA-1a) is strongly associated with the HLA-DRw3*0101 allele (Section 1.1.3).

Helper T cells express receptors which recognize peptides lodged in the HLA class II groove. It follows that processed peptides contain certain amino acids which contact the HLA class II molecule and a different set of amino acids which contact the T cell receptor. Thus, T cells recognize epitopes made up of linear amino acid sequences (in the HLA groove) rather than the conformational determinants which constitute the epitopes recognized by B cells and the antibodies they secrete. The interaction between antigen-presenting cells and helper T cells is facilitated by a series of cell adhesion and co-stimulatory molecules.¹ Once an antigen has been appropriately presented, helper T cells become activated and secrete cytokines such as interleukin-2 which are necessary for T cells to divide. These in turn release interleukins which cause B cells to differentiate into antibody-secreting clones (Figure 1.1).

In previously primed individuals, B cells can also take up antigen and present it, in the context of HLA class II, to helper T cells. Importantly, antigens displayed on the surface of B cells reflect the repertoire of antigens that B cells can recognize and select via specific membrane-bound antibodies. This means that helper T cells activate those B cells which have bound the antigen responsible for the initial activation of helper T cells (Figure 1.1). Again, the interaction between B cells and

Figure 1.1 (*cont.*)

(b) Cytokines involved in helper T cell activation include interleukin-1 (IL-1), IL-6, IL-12, IL-15 and tumour necrosis factor α (TNF α). IL-2 is also important in T cell proliferation. Cytokines involved in antigen-presenting cell activation (leading to increased expression of HLA class II and other molecules) include interferon γ (IFN γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4 and TNF β .

(c) Co-stimulatory molecules on activated helper T cells bind to their ligands on B cells; CD28 binds to CD80/CD86, CD40 ligand binds to CD40, LFA-1 binds to ICAM-1 or ICAM-3 and CD2 binds to LFA-3.

(d) Cytokines involved in B cell activation and differentiation include IL-4 and IL-6, respectively; these are characteristic of helper T cells (T_H2). Cytokines involved in the maintenance of T cell activation include IL-1 and IL-6.

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helper T cells involves several different receptor–ligand interactions and an exchange of soluble interleukins; HLA class II/peptide complexes and CD40 receptor molecules on B cells are ligated by T cell receptors and CD40 ligand molecules on helper T cells. This signals antigen-specific B cells to proliferate and differentiate into antibody-secreting plasma cells and memory B cells. The latter are responsible for the anamnestic response to rechallenge with antigen. Finally, the antigen-driven process of affinity maturation results in hypermutation of B cells and the secretion of antibody with increased affinity for antigen. Concurrent with the immune response, various mechanisms ensure the regulation of antibody production.¹

1.1.2 The maternal alloimmune response to fetal red cells

Human red cells express hundreds of different blood group antigens most of which have been reported to elicit a maternal alloimmune response (Chapter 2). Clinically, the most important antigen is D (Section 2.5.1): the antigen is relatively immunogenic, it is well developed early in gestation, a significant proportion of the Caucasian population is D negative, and the antibody is capable of causing fetal haemolysis.

In vitro, the immune response to peptides representing D-specific sequences results in the generation of cytokines characteristic of a helper T cell-dependent response.² In vivo, a primary immune response is followed by an anamnestic long-lived secondary immune response associated with the presence of circulating lymphocytes expressing D-specific membrane immunoglobulin.³ Helper T cells from different D-negative individuals appear to recognize and respond only to a limited number of peptides derived from regions distributed throughout the intracellular, transmembrane and extracellular regions of the D protein.⁴

The likelihood of a D-negative woman becoming immunized depends on several factors and these are reviewed below.

1.1.2.1 Dose of D-positive fetal red cells

Small volumes of fetal red cells enter the maternal circulation during most normal pregnancies and after most normal deliveries (Section 6.4).⁵ In the absence of antenatal prophylaxis, anti-D may be detected in sera from less than 1% of D-negative women bearing D-positive fetuses by the end of the third trimester.⁶ This implies that, during normal pregnancy, transplacental haemorrhage of sufficient red cells to elicit antibody production is rare.⁷ The volume of transplacental haemorrhage detected in the maternal circulation may be significantly greater at delivery, especially when the fetus and mother are ABO compatible. Thus, in the absence of Rh prophylaxis, about 16% of D-negative women become immunized as a result of their first D-positive ABO-compatible pregnancy; about one-half have detectable

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anti-D by 6 months after delivery and about one-half mount a brisk secondary response during a subsequent D-positive pregnancy indicating that a primary immunization had occurred.⁸ The incidence of alloimmunization increases to 31% following transplacental haemorrhage of over 0.1 ml fetal blood.⁹

Transplacental haemorrhage and alloimmunization may follow invasive procedures such as amniocentesis, fetal blood sampling, elective and spontaneous pregnancy loss, and some obstetric manipulations.^{10–12} These events and their associated risks of alloimmunization are discussed in more detail (Section 6.4).

1.1.2.2 Rh phenotype of the fetal blood

Red cells with the phenotype R_2r have been found to be more immunogenic than R_1r cells.¹³ This may be related to the density of D antigens on these cells; R_2r cells have 14 000–16 000 D antigens and R_1r cells have 9900–14 600 D antigens.¹⁴

1.1.2.3 ABO incompatibility

The relative rarity with which women become alloimmunized to D when the partner is ABO incompatible was first noted by Levine in 1943.¹⁵ In later studies, it was estimated that blood group A incompatibility between mother and fetus conferred 90% protection against immunization to D; blood group B incompatibility conferred 55% protection.¹⁶ Presumably, the protective effect is due to the ability of anti-A or anti-B to cause rapid intravascular destruction of fetal red cells.

1.1.2.4 Maternal HLA haplotype

Hilden et al. found the HLA-DQB1 allele *0201 in 18% of women with anti-D titres between 16 and 256 and in 85% of women with titres above 512, suggesting an association between this allele and a predisposition to form relatively high levels of anti-D.¹⁷ However, the molecular mechanisms responsible for this association have not been elucidated.

1.1.2.5 Fetal gender

Several studies have reported evidence which suggests that D-positive male fetuses elicit an alloimmune response more frequently than female fetuses. The male to female ratio in three studies was 1.4:1, 1.5:1 and 1.7:1.^{9,18,19}

1.1.3 The maternal alloimmune response to fetal platelets

Five of the glycoprotein receptors expressed in the platelet membrane have been shown to be polymorphic in Caucasian populations and so capable of eliciting a maternal alloantibody response leading to thrombocytopenia in the fetus (Section 12.2). Most cases of alloimmune thrombocytopenia are caused by maternal antibodies to the human platelet antigen-1a (HPA-1a). The HPA-1a antigen is caused

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by the presence of a leucine residue (rather than proline) at position 33 on the β subunit of the fibrinogen receptor (also called glycoprotein IIb/IIIa or CD41/61). In common with the alloimmune response to red cells, maternal anti-HPA antibodies are predominantly IgG1.^{20,21}

The pathogenesis of alloimmune thrombocytopenia differs from that of HDFN in that maternal sensitization to fetal platelet antigens often occurs in a first pregnancy, indicating that platelet antigens may be more immunogenic than red cell antigens.²² For example, transplacental passage of fetal glycoprotein IIIa may occur as early as week 14 of gestation.²³ Moreover, glycoprotein IIIa is found on syncytiotrophoblasts of the placental brush border.²⁴ Although glycoprotein IIIa is a type I transmembrane protein, its release into the maternal circulation might occur as the cells undergo apoptosis, perhaps during invasion of the endometrium.

The immune response to HPA-1a is under genetic control. Although only 5–10% of HPA-1a-negative women with HPA-1a-positive fetuses produce anti-HPA-1a (Section 4.2.1), the presence of the HLA-DRB3*0101 allele increases the risk of alloimmunization by a factor of 140.²⁵ Interestingly, the immune response to HPA-1b does not appear to be HLA restricted.²⁶ This implies that antigen-presenting cells require HLA-DRB3*0101 to present HPA-1a-derived peptides and that leucine at position 33 is involved in peptide binding in the HLA class II groove. This has been substantiated using an in vitro peptide-binding assay to show that leucine does indeed anchor the peptide to the HLA-DRB3*0101 molecule.²⁷ The proline form did not bind to the HLA-DRB3*0101 molecule which explains the rarity with which anti-HPA-1b antibodies are formed. The alloimmune response to another clinically important platelet antigen, HPA-5b, is also under genetic control.²⁸

1.1.4 The maternal alloimmune response to fetal neutrophils

There is a paucity of data on the nature of the maternal immune response to fetal neutrophils. It is well established that immunization may occur in first pregnancies so presumably fetal neutrophils can enter the maternal circulation during normal gestation.²⁹ The antibodies produced recognize granulocyte-specific antigens expressed on Fc γ receptor III (CD16), the complement receptor 3 (CD11b/CD18) and other, as yet unidentified, glycoproteins.^{30,31} Granulocyte antigens are fully expressed at birth and are discussed in detail later (Section 13.3.2).³²

1.2 Transfer of IgG to the fetus

The second critical step in the pathogenesis of the alloimmune cytopenias is the active transfer of IgG alloantibodies from the mother to the fetus. All four subclasses are actively transferred into the fetus via syncytiotrophoblast cells which

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express receptors for the Fc domain of IgG.^{33,34} These receptors are termed FcRn (Fc receptor neonatal) because they were first demonstrated in neonatal rodents where they transport IgG across the gut. Transfer of IgG across the placenta is slow until around week 24 and so HDFN before this time is rare. The rate of transfer increases exponentially during the second half of gestation until term when IgG levels in the fetus (approximately 15 g/dl) tend to exceed those in the mother (approximately 13 g/dl).³⁵

There is great variation between individual pregnancies. The rate of transport may be a major factor determining the severity of cytopenia in the fetus; in HDFN, it may be the rate-limiting step in the reaction between fetal red cells and maternal anti-D.^{36,37} There is some evidence that IgG1 is overrepresented in the fetal circulation early in gestation.³⁸ Also, IgG with relatively high levels of galactose is relatively abundant in the fetal circulation.³⁹ These forms of IgG may either be transported preferentially or catabolized relatively slowly in the fetus.

Recent studies have elucidated the molecular basis of IgG transport. The key to the process is the ability of FcRn to bind IgG with relatively high affinity at an acidic pH but with negligible affinity at the pH of blood (pH 7.4).⁴⁰ The FcRn molecule consists of an α chain which is homologous to HLA class-I molecules associated with β -2-microglobulin. The first step in the process is the nonspecific fluid-phase pinocytosis of IgG (like other plasma proteins) by the syncytiotrophoblasts. The IgG is then trafficked to apical vesicles where the acid pH causes it to bind to FcRn lining the vesicles. FcRn recognizes certain amino acids (isoleucine 253, histidine 310, histidine 435) at the interface between the C_H2 and C_H3 domains of IgG.⁴¹ Each IgG molecule has two heavy chains and may, therefore, bind two FcRn molecules. In this way, IgG binding results in FcRn dimerization which appears to be crucial for IgG trafficking. Bound IgG is then transported across the cells within the vesicles which eventually fuse with the basal cell membrane. This exposes the vesicle contents to neutral pH causing the IgG to dissociate from the FcRn and enter the fetal circulation.

1.3 The immune destruction of blood cells in the fetus

There are very few studies on the action of red cell or platelet antibodies in the fetus. The processes involved in the immune destruction of red cells and platelets have therefore been inferred from observations made in adults and from in vitro experiments. As a result of these studies, it is generally accepted that IgG alloantibodies opsonise blood cells causing their recognition by macrophages which express receptors for the Fc portion of IgG (usually abbreviated Fc γ R, where γ denotes specificity of the receptor [R] for IgG). Sensitized blood cells may then be destroyed by macrophages via a process termed extravascular lysis.

1.3.1 IgG and Fc γ receptors

The structure and function of IgG has been reviewed extensively⁴² and will be discussed only briefly here. IgG molecules are symmetrical, with two identical Fab fragments joined to one Fc fragment via a hinge region. The Fc fragment is comprised of two C_H2 domains and two C_H3 domains. Critical amino acid residues within the Fc region are responsible for interactions with Fc γ receptors on effector cells such as monocytes and macrophages.⁴² Four subclasses of IgG have been identified (IgG1, IgG2, IgG3 and IgG4). The subclasses show significant differences in the hinge region length which comprises of 5, 12, 62 and 12 amino acids in IgG1, IgG2, IgG3 and IgG4, respectively. The structure of the hinge region affects the flexibility of IgG. This flexibility, together with other subclass-related differences in amino acids at key positions within the Fc region, determines the relative ability of the IgG subclasses to interact with Fc γ receptors.

Effector cells such as monocytes and macrophages express different receptors for the Fc region of IgG. The structure and function of different Fc γ receptors have been reviewed in detail elsewhere.^{43,44} Macrophages express three classes of receptor for the Fc portion of IgG (Fc γ receptor I, Fc γ receptor IIa and Fc γ receptor IIIa). Fc γ receptor I binds human IgG with high affinity. Fc γ receptor IIa has low affinity for human IgG1 and IgG3. Fc γ receptor IIIa has an 'intermediate' affinity for IgG. The function of these Fc γ receptors in relation to red cell and platelet destruction is considered in more detail below.

1.3.2 The immune destruction of fetal red cells

Despite a wealth of *in vitro* data, it is unclear which of the Fc γ receptors are involved in the immune destruction of sensitized cells. *In vitro*, in the absence of fluid-phase IgG, Fc γ receptor I mediates the interaction between monocytes or macrophages and IgG anti-D-sensitized red cells.⁴⁵ However, a role for Fc γ receptor I *in vivo* seems unlikely because interactions between anti-D-sensitized red cells and this high affinity receptor are blocked by IgG concentrations several orders of magnitude lower than those found in plasma. Fc γ receptor IIa has a very low affinity for IgG1 and IgG3 and seems unlikely to play a role in the destruction of red cells sensitized with these subclasses.

There is indirect evidence that Fc γ receptor IIIa might play a role in the recognition of IgG-sensitized red cells *in vivo*. The administration of monoclonal antibodies to Fc γ receptor III increased the lifespan of IgG-sensitized red cells in chimpanzees and caused an increase in the number of circulating platelets in patients with autoimmune thrombocytopenia.^{46,47} The adherence of anti-D-sensitized red cells to adult splenic macrophages in cryostat sections is mediated, at least in part, by Fc γ receptor IIIa.⁴⁸

Although the molecular basis for the *in utero* destruction of IgG-sensitized red

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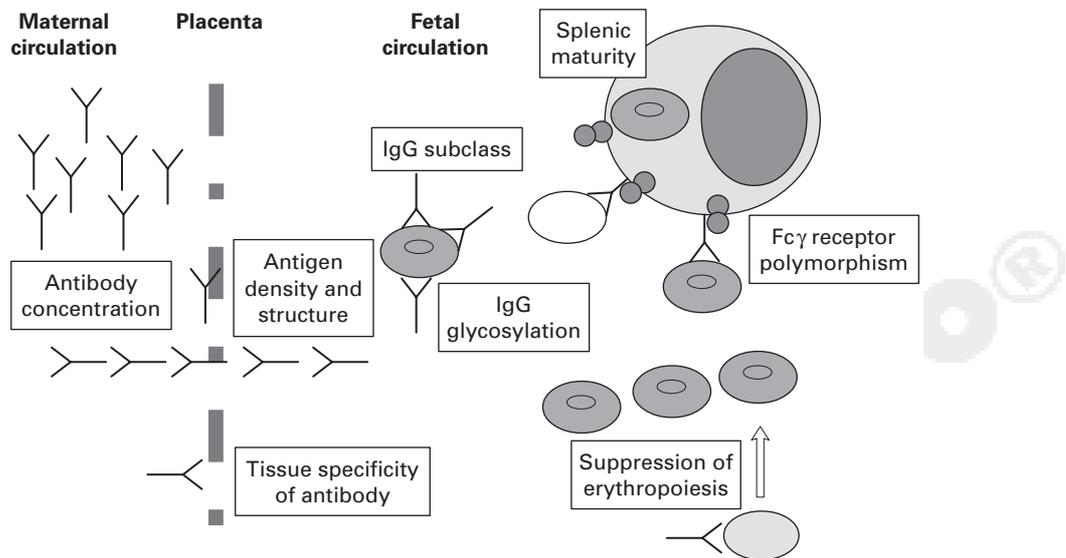


Figure 1.2 Some of the factors which determine the severity of haemolytic disease in the fetus.

cells remains to be fully elucidated, there is evidence to suggest that several factors may influence the extent of red cell destruction and, hence, disease severity. These are shown diagrammatically in Figure 1.2 and are considered below.

1.3.2.1 IgG glycosylation

IgG molecules contain a carbohydrate moiety within the C_{H2} domains. The carbohydrate helps to stabilize the tertiary structure of the molecule and is required for IgG to bind to $Fc\gamma$ receptors. The carbohydrate is heterogeneous and IgG from different individuals may contain different amounts of galactose. In vitro, the amount of incorporated galactose may affect antibody function. For example, monoclonal anti-D secreted under conditions which favour the incorporation of relatively high levels of galactose is more efficient at promoting lymphocyte-mediated lysis of red cells.⁴⁹ The relevance of these observations to the pathogenesis of HDFN is not yet established. Nevertheless, IgG galactose levels increase during pregnancy and it has been shown that anti-D in sera from pregnant women promotes lymphocyte-mediated haemolysis with greater efficiency than anti-D in sera from males.^{50,51}

1.3.2.2 IgG subclass

Sera from approximately one-third of women with anti-D contain only IgG1 anti-D while sera from most of the remainder contain a mixture of IgG1 and IgG3 anti-D.⁵² In vitro experiments using human monoclonal anti-D have shown that the

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phagocytosis and lysis of red cells by monocytes are promoted with greater efficiency by IgG3 anti-D than by IgG1 anti-D.⁴⁵ The longer hinge region of IgG3 may confer to the molecule the ability to span the gap between negatively charged red cells and monocytes with greater efficiency than IgG1. Although an association between IgG subclass and the extent of haemolysis in utero might be expected, studies using predominantly serological techniques have generated conflicting results. Some studies have failed to demonstrate any relationship,^{53–55} while others have reported an association between severe HDFN and IgG1 anti-D alone^{56,57} or the presence of both IgG1 and IgG3.^{52,58} The application of quantitative techniques should resolve these inconsistent results.

1.3.2.3 Antibody concentration

Once immunized, the concentration of antibodies in the mother influences the severity of haemolysis in the fetus; the concentration of anti-D in the fetal circulation reflects the concentration of anti-D in the maternal circulation.⁵⁹ The extent to which the antibody level determines disease severity and the ability of different assays to measure antibody concentration are considered in detail elsewhere (Section 8.3).

1.3.2.4 Antibody specificity

IgG antibodies to most red cell antigens have been reported to cause HDFN. The relationship between antibody specificity and clinical significance is reviewed in Chapter 2. However, it is possible to establish criteria which govern the potential of blood group antibodies to cause haemolysis in utero and these will be discussed briefly here. First, antibodies with the potential to cause haemolysis in utero recognize antigens which are restricted to the erythroid lineage; antibodies to antigens with a wider tissue distribution are absorbed by other fetal tissues and hence fail to localize on fetal red cells. The maturational specificity of blood group antigens may also affect the pathogenesis of HDFN. Thus, antibodies to antigens of the Kell system, which are expressed at an early stage of erythroid development, may cause anaemia by suppressing erythropoiesis or by promoting the immune destruction of erythroid precursor cells.^{60,61} The second criterion is antigen density. Some antigens (for example, Rh antigens) are well expressed early in embryonic development. Other antigens (for example, Lutheran antigens) are poorly expressed on fetal cells. Antibodies to antigens which are expressed at low density on fetal red cells are unlikely to cause HDFN.⁶² A third criterion may be antigen structure. As a general rule, antibodies to antigens which are relatively distant from the lipid bilayer more efficiently promote the recognition of red cells by monocytes.⁶³ Thus, IgG anti-K is more effective at promoting the adherence, phagocytosis and lysis of red cells than IgG anti-D.⁵⁴ IgG2 anti-A promotes Fcγ receptor IIa-mediated rec-