# Immunology in the Fetus and Neonate

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## 12.1 Introduction

Immune system development is dependent on precise interactions between developing specialized tissues and organs as well as hematopoietic cell development from a common stem cell. Once mature, the innate immune system is the immediate response to infection while the adaptive immune system generates specific long lasting responses to antigen. Although the innate immune system includes barriers such as skin and cilia, the focus of this chapter is on the hematopoietic cells of the innate and adaptive immune system and the tissues that support their development. The major function of the immune system is to protect the body from infections. When the immune system becomes dysregulated, autoimmune diseases and allergy result. When the immune system does not develop normally, primary immunodeficiency results, leaving the individual susceptible to infection. An awareness of immunological defects in fetuses and neonates may provide an explanation for fetal and neonatal deaths. Whether caused by genetic factors like primary immunodeficiency, or maternal factors such as passively transferred autoantibodies, knowledge of these conditions may enable successful outcomes for future pregnancies and optimal treatment for future affected infants.

## 12.2 Development of the Immune System

At between 4 and 6 weeks of gestation, the AGM (aorta-gonad-mesonephros) region forms in the mesodermal tissue along the dorsal ridge. Hematopoietic stem cells, as defined by CD34 expression and their ability to differentiate into all blood lineages, emerge from this region. Over time these cells migrate to the bone marrow from where definitive hematopoiesis occurs [1]. This process is driven by the transcription factor BMP4 which regulates the expression of c-KIT which is required for the maintenance of multipotential hematopoietic stem cells from which all blood cells develop [2].

### 12.2.1 The Innate Immune System

#### 12.2.1.1 Cells

The cells of the innate immune system are the first cells to respond to infection. They develop from common hematopoietic stem cells in the AGM and are detectable in the liver, thymus and spleen from 8 weeks of gestation onwards. A common granulocyte monocyte progenitor differentially responds to the transcription factors C/EBP, Gfi1 and PU.1 to develop into neutrophils and eosinophils, or monocytes that can develop further into macrophages. Around 15 weeks of gestation both neutrophils and monocytes can be detected in the fetal circulation [3]. Major histocompatibility Class II expression is detectable on fetal blood samples from 15 weeks onwards. After week 32, hematopoiesis primarily occurs in the bone marrow from where neutrophils, eosinophils, and monocytes develop and migrate [4]. At birth neutrophil numbers and function are similar to those in adults. However, preterm and small for age infants may be neutropenic for the first week of life and degranulation is also impaired in these infants; these should normalize at the end of the first week of life. In addition, chemotaxis in neonates is approximately 50% of that seen on older children and adults; however, neonate neutrophils demonstrate normal phagocytic and bactericidal activity [5].

#### 12.2.1.2 Complement and Other Serum Proteins

In addition to the development of immune cells, there are serum proteins that are essential components of the innate

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immune system. The complement system is comprised of 3 pathways (classical, alternative and lectin) and over 50 proteins. It operates in a steady state but also becomes activated as part of the innate defense system. To control inflammation and immune cell activation there are a number of regulators of the complement system [6]. During pregnancy, in addition to complement being synthesized by the liver of both the mother and the fetus, it is also made by the placenta. C3, a key complement component, can be made by the uterus and appears to be important for implantation prior to placental development [7]. Although C3 can be detected as early as 5½ weeks gestation, complement activity in the newborn is about 50% of maternal complement activity [5]. This may be a mechanism of protecting both the mother and fetus from complement activity.

Deficiencies in complement components are well described. C3 deficiency is a rare but well described complement deficiency. To date, there are no literature reports of women with C3 deficiency having a successful pregnancy [8]; this may be because C3 deficiency is rare and individuals with C3 deficiency frequently die of overwhelming infection before reaching adulthood. The effect of complement mutations in the embryo and on embryonic and fetal development as well as pregnancy outcome has not been well characterized.

Complement components are also found in breast milk and can cause opsonisation via the alternate pathway, providing extra protection for the breastfed infant. As well as complement, other innate immune factors are also present in breast milk. These include a number of anti-bacterial enzymes and compounds such as lysozyme which can disrupt bacterial cell walls, and lactoferrin which inhibits the growth of bacteria [9].

### 12.2.2 The Adaptive Immune System

Unlike the innate system, the adaptive immune system is specific for the antigen encountered. This specificity is achieved by the antigen receptors of T cells and B cells, which are the main cells of the adaptive immune system. White blood cell progenitors that give rise to these cells of the adaptive immune system are derived from stem cells. These stem cells develop during embryogenesis in the erythropoetic islands in the yolk sac. At approximately 6 weeks of gestation, progenitor cells in the fetal liver begin to produce blood cells. The liver remains the main source of blood cell production from the 11th week into the early months of gestation. From approximately 4 months, this production begins to move to the bone marrow, and by 6 months and for the rest of the fetus's life, this is the source of hematopoiesis.

#### 12.2.2.1 T Cells

The thymic epithelium develops by 8 weeks of gestation, and T cell precursors which migrate to the thymus can be detected there from this time [10]. These early T cell precursors lack expression of either the CD4 or CD8 co-receptor and are termed 'double negative' T cells. In the thymus, T cells rearrange their antigen receptor (T cell receptor or TCR), and express CD3 on their surface. They then undergo 'positive selection' to ensure that their TCR is capable of recognizing antigen, and upregulate expression of both CD4 and CD8 to become 'double positive' i.e. CD4+ and CD8+ T cells. They then undergo 'negative selection' to ensure that their TCRs are not self-reactive. At this stage they downregulate one of their co-receptors to become 'single positive' i.e. they become either CD4+ or CD8+ T cells [11]. T cells are found in the periphery at a ratio of 2:1 CD4:CD8, and also in lymph nodes, the spleen, and other lymphoid organs. If T cells are unable to successfully rearrange their antigen receptor to form an alpha/beta receptor, they can form a TCR using the gamma/delta chains; although gamma/delta T cells do not have the same range of antigen specificity as alpha/beta T cells.

T cells can be detected in the periphery as early as 15 weeks [12]. However, they show reduced effector function until later in gestation. At term, although T cell numbers are high compared to adults, cytotoxic T cell function and production of cytokines such as Interferon gamma and Tumor Necrosis Factor alpha is still low. Full cytotoxic activity and cytokine production do not reach adult levels until 2 months of age [13].

#### 12.2.2.2 B Cells

Similarly to T cells, B cells precursors begin to rearrange their antigen receptors (B cell receptors or BCR) at around 7–8 weeks of gestation [14] and B cells can be found in the periphery at around 12 weeks of gestation [15]. At birth, IgM antibodies are detectable to specific proteins but not to carbohydrate antigens, but antibody detection of carbohydrates matures by the age of 2 years [16]. T-cell 'help' is required for B cells to proliferate and differentiate into plasma cells, to 'class switch' and produce classes of antibody other than IgM. This help is achieved by binding of CD40L on the surface of activated T cells to CD40 on the surface of B cells, and production of cytokines such as IL-4 and IL-21 by T cells which influence the B cell [17]. In the first few months of life there is poor T-cell help for B cells as Th2 cytokines (including IL-4 and IL-21) are low [18], and there are low CD40L levels on T cells [19]. This generally reaches adult levels by approximately 6 months of age [20].

#### 12.2.2.3 Immunoglobulins

Of the five classes of immunoglobulins IgM, IgA and IgG have been robustly studied in fetal development. IgM is the

first immunoglobulin to appear at 20 weeks gestation [21]. IgA is only detected in term infants and increases with age. Although IgA is not maternally transferred, it is present in high concentrations in colostrum [22], and breast milk, and resists digestion in the neonatal gut to act to neutralise viruses and bacteria.

IgG is the only immunoglobulin to cross the placenta, and transport occurs via specific receptors in the trophoblast. Transfer begins at about 12 weeks of gestation, but the majority of IgG is transferred after 32 weeks of gestation. As a result, extremely premature infants and neonates with reduced fetal growth due to poor placental function will have significantly reduced circulating maternal IgG at birth and are therefore susceptible to bacterial infections [23].

The neonate does not make detectable levels of immunoglobulin until 15 weeks of life [22]. There is a strong linear correlation between birth weight, age of gestation and IgG levels that persists until 34 weeks gestational age [22]. In term infants, IgG levels are 75% of maternal levels [24]. Over the first six months after birth, the maternal IgG levels in the infant decrease as the maternal IgG is catabolized. As the infant begins to make their own immunoglobulin, there is a resulting period of relative IgG deficiency in the first 6 months of life, until T-cell help for immunoglobulin class switching increases. IgG levels approach adult levels by 4 years of age (Fig. 12.1).

Because of maternal immunoglobulin transfer, specific antibodies, either autoantibodies e.g. anti-dsDNA or anti-microbiological antibodies e.g. anti-toxoplasma will be of maternal origin for the first three months of life and partly maternal for the next three months of life. If primary immunodeficiency is a possible diagnosis, the ability of the infant to make specific antibodies needs to be assessed. As specific IgG responses will be of maternal origin, measurement of isohemagglutinins can be performed, as this is an IgM-based assay used in blood group typing [25]. It is routinely available in hematology laboratories and, as these are IgM antibodies which will be of fetal origin, can easily determine if the infant can make functional antibodies. A key indication that an infant may have antibody deficiency is the inability to easily blood group an infant [26].

## 12.3 Diseases Resulting from Primary Immune Deficiency

### 12.3.1 Disease with Abnormal Organ Development

Patients with abnormal immune system organ/tissue development are susceptible to particular infections as they lack specific cells that normally develop in the missing/abnormal organ/tissue.

#### 12.3.1.1 Asplenia

Asplenia is most commonly diagnosed when patients have visceroarterial heterotaxy, as the cardiac and vessel abnormalities result in clinicians considering asplenia (see also Chaps. 20 and 27). Less frequently (~1 in 1 million live births) isolated asplenia occurs. These patients fail to develop a spleen but no other abnormalities are identified. Isolated asplenia can be inherited as an autosomal dominant disease (*RPSA* is one gene that has been identified), an X-linked disease (2 genes have been identified: *GJA1* (connexin 43) and

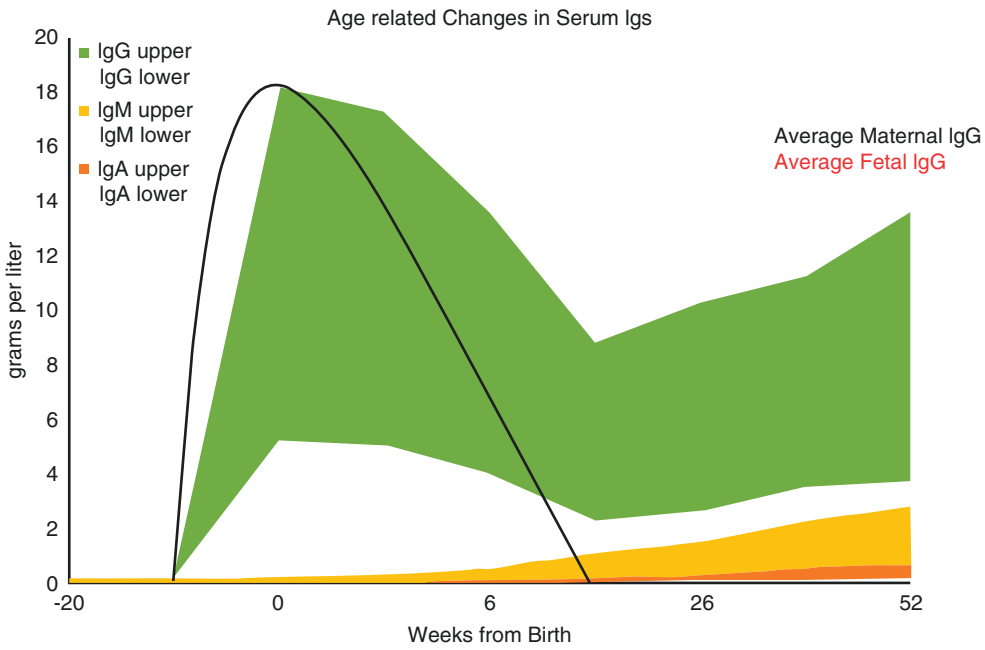


**Fig. 12.1** Immunoglobulin changes from 20 weeks gestation through the first year of life. Green represents the normal range for IgG. The black line indicates maternal transfer and the red line neonatally-produced IgG. Yellow represents the normal range for IgM and orange represent the normal range for IgA. (Illustration by Nathaniel Gilmour)

*ZI63*) and as an autosomal recessive disease [27]. Splenic lobules start to develop between weeks 15 and 17 of gestation; lymphoid colonization starts to occur at week 18 and by week 23 of gestation primary follicles are visible in the spleen [28]. The lack of a spleen results in abnormal B cell development. Patients with asplenia are susceptible to particular bacteria such as pneumococcal infections. Infants are usually protected by transfer of maternal IgG until six months of age after which they start to develop infections. As soon as asplenia is diagnosed, patients should commence lifelong antibiotic prophylaxis and undergo vaccination with conjugate vaccines for pneumococcal disease and meningitis. As isolated asplenia may be both sporadic and familial, it is essential to diagnose and ensure genetic analysis is undertaken so that family members can be screened and treated as needed. Unfortunately the diagnosis of asplenia is frequently made on post-mortem examination.

#### 12.3.1.2 Absent Tonsils

As B cells comprise about 65% of tonsillar tissue, patients with genetic defects resulting in absent B cells have hypoplastic secondary lymphoid organs such as tonsils [29]. As fetuses are protected in utero from infection and usually for the first six months because of maternal IgG transfer, infants lacking B cells do not usually present with illness until 6 months of age or later. Due to the lack of B cells, these patients fail to make immunoglobulins and are susceptible to bacterial and enteroviral infections. Any fetus/infant lacking tonsils (palatine or adenoids) should have genetic analysis for B cell immunodeficiency undertaken [30]. Several genetic diseases where patients lack B cells have been identified including Bruton's Tyrosine Kinase (BTK) deficiency. BTK deficiency, also known as X-linked agammaglobulinemia, is an X-linked condition identified by Bruton in 1952 who demonstrated that patients lacking B cells have absent tonsils [31]. Patients lacking B cells are treated with replacement immunoglobulin and have a normal life span and quality of life as long as they are compliant with treatment. Some patients lacking secondary lymphoid tissue and B cells also lack T cells and have severe combined immunodeficiency (SCID) or other combined immunodeficiencies (CID) (see below).

#### 12.3.1.3 Athymia/Small Thymus

As discussed, the thymus is the site of T cell development following hematopoiesis of T cell precursors in the bone marrow. In the absence of a thymus, normal T cell development cannot occur. Absence of a thymic organ may appear on chest X-ray, and may also be revealed at autopsy. Identification of thymic aplasia/hypoplasia with the clinical features described below should prompt further investigation for these conditions. A number of causes of athymia/hypothymia have been identified and are discussed here (see also Chap. 27).

##### Di George Syndrome

Di George syndrome (DGS) is a clinical term describing a collection of features including cardiac, palatal and renal abnormalities, endocrine abnormalities in particular hypoparathyroidism and hypocalcemia, and immune dysfunction which can range from mild to severe [32]. DGS has an incidence of 1 in 4000 births [33], and is caused by abnormal development of the third and fourth pharyngeal pouches during embryogenesis, which can lead to thymic aplasia/hypoplasia.

It is caused primarily by 22q11.2 chromosomal micro deletion in which a 3 Mb section of chromosome between two regions of low copy number repeats is lost, likely through homologous recombination. This is generally a de novo phenomenon (90–95%), but the deletion may be inherited from an affected parent [34]. DGS may also rarely be caused by point mutations in the *TBX1* gene (an important regulator of transcription factors involved in embryonic development), which is contained in this 3 Mb region [35].

There is considerable overlap between DGS and Velocardiofacial syndrome (VCF, also caused by 22q11.2 deletion), to the extent that many now refer to DGS and VCF simply as 22q11 deletion syndrome [36]. However, the term DGS will be used throughout this section.

DGS may also rarely (1 in 200,000) be caused by a 10p chromosomal deletion, although the clinical features of renal abnormalities, developmental retardation, and sensorineural deafness are more prevalent in 10p deletion compared to 22q11.2 deletion [37]. Other causes of DGS include maternal/gestational diabetes [38], and fetal alcohol syndrome [39], as well as fetal exposure to toxins e.g. retinoic acid [40].

DGS may be diagnosed by clinical examination and detection of anomalies e.g. cardiac defects by echocardiography. A diagnosis of 22q11.2 deletion can be confirmed by CGH array or FISH. Prenatal diagnosis may be made using CVS/or amniocentesis where DGS is suspected (e.g. anomalies detected by fetal echocardiogram or known affected parent).

Immune dysfunction in DGS can range from a mild lymphopenia, to a T cell deficient SCID-like (see next section) presentation. The majority of patients with DGS have a milder immune dysfunction with some increased susceptibility to infection, and autoimmunity arising as a result of immune dysregulation through a lack of normal T cell development in the thymus. The percentage of cases with a complete T cell deficiency (sometimes referred to as 'complete' DGS in contrast to 'partial' DGS' in which T cells are present) is likely underestimated as immunophenotyping is not

always carried out in these patients. Nevertheless, approximately 1% of DGS cases are T cell deficient. These patients present with a T cell count of less than 50, but normal numbers of B and NK cells. Any T cells present may be present due to maternal fetal engraftment, or oligoclonal expansion of memory T cells [41]. Newborn screening using TRECS (available in many countries and entering a pilot phase in the UK at the time of writing) will identify neonates with 'complete' DGS (as well as other causes of T cell deficiency see below). These children are at risk of the same opportunistic infections as those with SCID, and are likely to die in infancy without corrective treatment; thymic transplantation is the preferred therapeutic option, although at the time of writing is only available in two specialist centers worldwide [42].

#### CHARGE Syndrome

CHARGE syndrome shares a number of clinical features with DGS, including immune abnormalities. CHARGE syndrome is named for its clinical features of Coloboma of the eye, Heart defects, Atresia of the choanae, Restriction of growth and/or development, Genital hypoplasia, and Ear abnormalities (external, middle and inner including deafness). It has an incidence of approximately 1 in 8000 births [43].

CHARGE is caused by defects in the *CHD7* gene. Like DGS, it arises primarily de novo (>97%), but can also be inherited in an autosomal dominant manner [44]. Similar to *TBX1*, *CHD7* is a regulator of other transcription factors important in development, and is essential for the developmental of the migratory neural crest in embryonic development. CHARGE syndrome is diagnosed by clinical examination and by molecular analysis of the *CHD7* gene and CGH array to exclude chromosomal deletions associated with similar disorders e.g. DGS [45].

T cell deficiency is also found in CHARGE syndrome. In one cohort study, thymic aplasia was found in 50% of the cohort, with low or absent T cell numbers in 80% of the whole cohort. An associated hypogammaglobulinemia was also noted, highlighting the role of T-cell help in immunoglobulin production [46].

#### Severe Combined Immune Deficiency (SCID)

Severe Combined Immune Deficiency (SCID), as the name suggests, is a life-threatening condition with a combination of low/absent T cells with or without absent B cells, NK cells, or an absence of all three immune cell types. The absence of T cells results in thymic and splenic hypoplasia. SCID is a medical emergency and early identification is vital to enable appropriate immediate treatment, treatment of the underlying genetic defect and to prevent mortality; the condition is otherwise usually fatal within the first year of life.

Children with SCID generally present in the first few months of life with severe, persistent infections, usually caused by opportunistic organisms, and a failure to thrive. Typical infections include Candida albicans, Pneumocystis jirovecii, and cytomegalovirus (CMV). Children who present with such features should be investigated for immune deficiency. As a first step the lymphocyte count should be measured; a lymphocyte count of less than 1000 in a child under the age of 1 should be presumed to be due to SCID until proven otherwise. A low lymphocyte count is sometimes overlooked, but can provide a vital first clue to prompt further investigation. It is important to note that patients with T negative B positive SCID often have normal lymphocyte counts as the B cells expand in the absence of T cells. Further immune phenotyping shows low/absent T cells alone, or with low/absent B cells, NK cells, or both. Any B cells present will be unable to receive T-cell help to class switch so immunoglobulins are low/absent [47].

SCID can be caused by an ever-increasing number of genetic defects. Immune phenotyping can provide a clue as to the potential affected gene, thus enabling more rapid genetic analysis as only a handful of genes are examined for mutations. The more common genetic causes of SCID, along with their immunophenotype, a brief description of the pathogenesis, and any notable clinical features are detailed in Table 12.1.

A diagnosis of pediatric HIV from maternal transmission should also be excluded in patients with opportunistic infections or lymphopenia. HIV is now much less common than was previously the case as a result of antenatal screening and support for HIV positive mothers, although cases do still occur.

### 12.3.2 Disease with Abnormal Cell Function

Although lacking obvious organ abnormalities, some immunodeficiencies may present *in utero* due to specific abnormal cell functions. The best characterized of these are primary/familial hemophagocytic lymphohistocytosis (FHL), IPEX, and Wiskott-Aldrich syndrome (WAS).

#### 12.3.2.1 Familial HLH

Mutation in four genes have been shown to be the cause of FHL: *PRF* (perforin), *UNC13B* (munc 13-4), *STX11* (syntaxin 11) and *STXBP2* (munc 18-2). In addition, patients with Chediak Higashi Disease (mutations in *Lyst*) and Griscelli's syndrome (mutations in *Rab27a*) can present with hemophagocytic lymphohistiocytosis (HLH) alongside pigmentation defects. FHL is frequently triggered by infections including Epstein Barr Virus. FHL is usually diagnosed in

**Table 12.1** Common genetic causes of SCID, immunophenotype, pathogenesis, and notable clinical features

| Disease name | Immunophenotype | Possible gene(s) | Inheritance pattern | Pathogenesis | Other features |
|---|---|---|---|---|---|
| IL7Rα deficiency | T-B+NK+ | *IL7R* | Autosomal recessive | Absent signaling through the IL-7R; required for T cell development | |
| Winged helix deficiency | T-B+NK+ | *FOXN1* | Autosomal recessive | Lack of a transcription factor required for thymic development | Abnormal thymic epithelium, congenital alopecia, nail dystrophy, neural tube defect |
| Di George | T-B+NK+ | Deletion 22q | Autosomal haploinsufficiency | As above | Absent thymus, hypoparathyroidism, cardiac malformations, cleft palate, facial dysmorphism |
| Di George | T-B+NK+ | *TBX1* | autosomal haploinsufficiency | As above | Absent thymus, hypoparathyroidism, cardiac malformations, cleft palate, facial dysmorphism |
| Charge | T-B+NK+ | *CHD7, SEMA3E* | autosomal haploinsufficiency | As above | Coloboma, heart anomaly, choanal atresia, intellectual disability, genital and ear anomalies; CNS malformation; |
| XSCID | T-B+NK- | *IL2RG* | X-linked | Absent signaling through cytokine receptors which use the common gamma chain, in particular IL-2 receptor; signaling through this is required for T cell and NK cell development | |
| JAK3 deficiency | T-B+NK- | *JAK3* | Autosomal recessive | Absent downstream signaling from cytokine receptors; as above | |
| RAG SCID | T-B-NK+ | *RAG1/2* | Autosomal recessive | Failure of VDJ recombination in TCR and BCR formation due to lack of RAG activity leading to lack of T and B cell production | |
| Artemis | T-B-NK+ | *DLRE1C* | Autosomal recessive | Failure of DNA repair of RAG 1/2 activity | |
| Radiation sensitive SCID | T-B-NK+ | *LIG4, NHEJ1, PRKDC* | Autosomal recessive | Failure of double stranded DNA break repair so TCR and BCR rearrangements fail | Microcephaly and LIG4/ NHEJ1 have facial dysmorphism |
| ADA deficiency | T-B-NK- | *ADA* | Autosomal recessive | Lack of ADA enzymatic activity leading to build up of toxic metabolites | Hypoplastic secondary lymphoid organs, Neurological abnormalities |
| Reticular dysgenesis | T-B-NK- | *AK2* | Autosomal recessive | Absence of stem cells | Pancytopenia, Bilateral sensorineuronal deafness |
| MHC II deficiency | Low CD4 T cells (absent HLA-DR expression) | *RFXANK, CIITA, RFX5, RFXAP* | Autosomal recessive | Failure of antigen presentation in thymus and periphery so CD4+ T cells fail to develop | |
| MHC I deficiency | Low CD8+ T cells (for TAPs absent MHC I expression) | *TAP1, TAP2, TAPBP, ZAP70* | Autosomal recessive | Failure of antigen presentation in thymus and periphery so CD8+ T cells fail to develop | |

*CNS* central nervous system

the neonate or older infant with 70–80% presenting before the age of 2 years [41]. However, there are a number of well documented cases of FHL presenting *in utero* as early as 24 weeks of gestation with hydrops fetalis being the most common presentation [48–50]. Infectious triggers seem to be uncommon in *in utero* and neonatal HLH. Heeg et al. reviewed 25 cases from the German HLH registry presenting between 24 weeks of gestation and 8 days of life and failed to identify an infection in any of these infants [51]. Patients presenting *in utero* and in the first days of life have a significantly worse outcome that those presenting later. Diagnosis is often made at post mortem examination. As the outcome in these infants is poor, diagnosing FHL as soon as possible enables optimal treatment (immune suppression followed by hematopoietic stem cell transplant [HSCT]) as well as family counseling for future pregnancies.

#### 12.3.2.2 IPEX

Although the majority of cases present in the first year of life, to date there have been 7 cases of IPEX (Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked) described in fetuses. IPEX is a rare X-linked condition caused by mutations in *FOXP3*. *FOXP3* is a transcriptional regulator required for regulatory T cell development, and patients with IPEX lack regulatory T cells. Because of the lack of regulatory T cells, these patients (all male as an X-linked condition) develop autoimmune diseases including diabetes mellitus, severe enteropathy and eczema. Infants presenting *in utero* are described as having hydrops fetalis, intrauterine growth restriction and frequently are born prematurely. A recent paper also describes echogenic bowel and echogenic debris as well as skin desquamation in two IPEX fetuses; these finding were detected by ultrasound [52]. One infant died at delivery and post mortem histological analysis of the skin showed infiltration of lymphocytes, histiocytes, eosinophils, and multinucleated giant cells consistent with a dysregulated immune system. Curative treatment for IPEX is HSCT.

#### 12.3.2.3 Wiskott Aldrich Syndrome

At birth thrombocytopenia may be recognised as a result of trauma the infant has undergone during delivery and, as a result of bleeding, thrombocytopenia is diagnosed. It may also be detected on an incidental full blood count performed for other reasons such as infection or because of gastrointestinal bleeding. A number of conditions cause neonatal thrombocytopenia including sepsis, necrotising enterocolitis, congenital infection and metabolic diseases. Low platelets may also be the result of maternal anti-thrombocyte antibodies or placental insufficiency. Approximately 1% of neonatal thrombocytopenia is caused by inherited conditions [51]. In these rare cases, a blood film examining platelet size can be diagnostic as patients with Wiskott-Aldrich syndrome (WAS) have small platelets, thrombocytopenia, as we as being prone to bacterial and viral infections including CMV. Autoimmune disease is frequently observed in the older infant with WAS. If a clear explanation for neonatal thrombocytopenia is not rapidly identified, genetic analysis is suggested to distinguish genetic from secondary causes.

### 12.3.3 Disease with Abnormal Skeletal Development

#### 12.3.3.1 Cartilage Hair Hypoplasia

In addition to disease that primarily affect cells of the immune system, several multisystemic diseases that affect the skeleton as well as the immune system may be diagnosed *in utero* or at birth. Cartilage hair hypoplasia (CHH) is a syndromic condition with abnormalities of the skeleton and hair as well as the immune system. It is an autosomal recessive disease caused by mutations in *RMRP*. Not all features are present in all patients. Because of the short-limbed dwarfism and characteristic X-ray finding, patients are frequently diagnosed as neonates and some are diagnosed in utero via ultrasound. [53]. As the immunodeficiency is highly variable ranging from slightly low T cells to absent T cells more consistent with a diagnosis of SCID, infants suspected or diagnosed with CHH should have immunophenotyping undertaken to measure their B, T and NK cells as well as their naïve T cells. If these are low, then a referral to a pediatric clinical immunologist should be made.

#### 12.3.3.2 Hyper IgE Syndrome

Autosomal dominant Hyper IgE Syndrome (HIES) frequently presents at birth or in the neonatal period with a pustular rash, and on skin biopsy eosinophils are commonly present. Patients with autosomal dominant hyper IgE syndrome, previously known as Job syndrome, are susceptible to recurrent infections, particularly Staphylococcus aureus as well as being prone to trauma fractures (osteoporosis is observed; although the degree of osteoporosis does not correlate with fracture occurrence), they retain their primary teeth and with age develop a unique facial appearance. Patients with HIES have elevated IgE levels (>2000 IU/μl) and eczema is common although allergies are not. Autosomal dominant HIES is caused by mutations in *STAT3*. There are also autosomal recessive forms of HIES but these do not have the associated skeletal and connective tissue abnormalities [54]. Patients with rash and an elevated IgE level in the neonate should have the genes causing HIES (*STAT3* and *DOCK8*) sequenced.

## 12.4 Diseases Resulting from Secondary Immune Deficiency

Maternal malnutrition affects the growing fetus and has an impact on the immune system of the infant. Infants born to malnourished mothers have lower IgG levels, as malnourished woman have lower IgG levels resulting in less IgG transferred across the placenta. Malnourished mothers also have less IgA in their breast milk. In addition, deficiency of certain micronutrients affects fetal immune system development. Infants lacking zinc during gestation have smaller spleens and thymuses, lower immunoglobulin levels and poor T cell activation. Vitamin A and D have been shown to be required for regulatory T cell development [55]. Retinoic acid has also been shown to be essential for the development for the spleen [56]. Therefore it is essential to assess maternal nutrition at the both the macro- and micro nutrient level.

### 12.4.1 Diseases Resulting from Maternal Autoimmune Disease

Women with Systemic lupus erythematosus (SLE) and antiphospholipid syndrome are at increased risk of miscarriage, early fetal death as well as fetal growth restriction and preeclampsia. These are all the result of immune complex formation and laboratory markers in maternal plasma include decreased C3 and C4 levels as well as increased sC5b-9; antiphospholipid antibodies are also present [8].

Women with SLE, as well as those with other connective tissue diseases, may have anti-Ro and anti-La extractable nuclear antigen antibodies. In the fetus these antibodies, transferred across the placenta from the mother, can cause autoimmune congenital heart block (CHB). CHB occurs in fetuses with anatomically normal hearts with heart block. CHB is usually diagnosed *in utero* but may be diagnosed at birth or in the neonatal period. Anti-Ro or anti-La antibodies in a mother with a fetus with CHB is diagnostic of CHB. These antibodies can also be measured in the infant. CHB has a high mortality with 70% of affected fetuses dying in utero [57].

Infants born to mothers with either hyper- or hypothyroidism are more prone to a number of complications including sepsis, respiratory distress syndrome, transient tachypnea and apneas [58]. In addition, congenital hypothyroidism was a significant cause of intellectual impairment until it became part of routine newborn screening [59]. Fetal hyperthyroidism may occur at 18 weeks in severe cases and more commonly from 24 weeks of gestation onwards [60]. Thyroid receptor stimulating antibodies are the marker of Graves disease. It is essential that appropriate thyroid hormone replacement occurs during pregnancy in these patients to avoid hyper- or hypothyroidism in the fetus and associated complications.

Congenital arthrogryposis can also be seen in utero, and neonatally, in infants born to mothers with myasthenia gravis. This leads to feeding and respiratory difficulties, though not all are affected. The condition resolves spontaneously in the majority with only supportive care usually required [61].

## 12.5 Diagnosis of Primary Immunodeficiency In Utero, at Birth and Post-mortem

Diagnosis of Primary Immunodeficiency (PID)/Inborn errors of immunity (IEI) may be made in utero through standard genetic screening of chorionic villus samples (CVS) or, in some cases, cell-free fetal DNA. For families where a genetic condition is suspected but no genetically confirmed diagnosis has been made, fetal blood sampling is possible. At birth, newborn screening by TRECS is possible, along with immunophenotyping and genetic analysis (see below).

### 12.5.1 Fetal Blood Testing

For fetuses where abnormalities of the immune system are possible, where a genetic etiology has not been identified and where the mother would terminate an affected fetus or where post mortem blood is obtained from a fetus or preterm infants, the presence or absence of cells and proteins can be analyzed by flow cytometry and this can diagnose SCID and other PIDs. Integrins (CD18) involved in neutrophil migration and absent in leukocyte adhesion deficiency (LAD); these can be detected from 15 weeks gestation. From 15 weeks onward HLA-DR protein can be measured. If present, MHC II deficiency is excluded. From 17 weeks gestational age, T cells may be detected in a fetal blood sample. These are summarized in Table 12.2. T and NK cell percentages vary with fetal age with T cells being approximately 40% in the fetus and increasing slightly in the neonate; NK cell in the fetus are about 5% of the lymphocytes and these increase to approximately 12% in the neonate [62]. If FHL is suspected, perforin expression can be analyzed in samples from fetuses of 23 weeks or more gestation. Otherwise, the presence or absence of cells and proteins can be analyzed after birth (see below).

### 12.5.2 New-Born Screening

Newborn screening for SCID has been implemented in many countries throughout the world and has been shown to be a robust way of identifying infants with absent T cells [63]. Infants with absent T cells have Severe Combined Immunodeficiency (SCID), a T cell development disorder caused by lack of a functional thymus or T cell lymphopenia of unknown cause. The assay uses a punch from the dried

**Table 12.2** Cell type, week of gestation and markers to identify specific diseases

| Cell type | Week of gestation detectable in fetal blood | Markers | Disease association |
|---|---|---|---|
| Monocyte | 15 | DR | MHC II deficiency |
| Neutrophil | 15 | CD18 and CD11a,b, c | LAD1 |
| T cell | 17 | CD2, CD3 and CD7 | SCID |
| B cell | 20 | Cd19 and Cd20 | Agammaglobulinemia |

*LAD1* Leukocyte adhesion deficiency-1; SCID severe combined immune deficiency

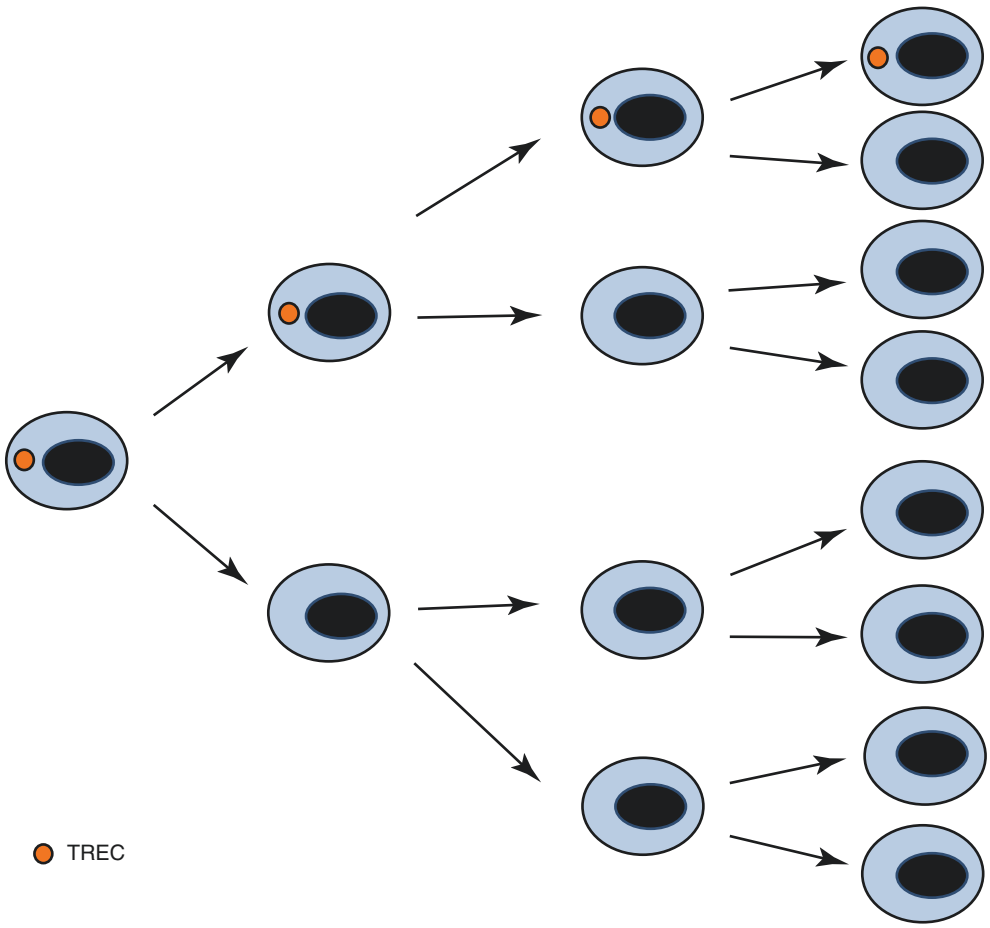


**Fig. 12.2** Dilution of TRECS (red circle) with each cell division (Illustration by Nathaniel Gilmour)

blood spot (Guthrie) card and quantitative PCR to detect TRECS (T cell ring excision circles). TRECS are formed when T cell receptors are rearranged in the thymus and are a residual circular piece of DNA that remains in the cell. Every time the cell divides, one daughter cell receives the TREC and one cell does not, so as T cell expansion occurs TRECS are diluted out (Fig. 12.2). As most T cells in infants are recent thymic emigrants and have minimal T cell expansion, TRECS should be high, and low/absent levels of TRECs are specific for T cell immunodeficiency. Based on TREC screening on blood spots, reviewing the first 11 states in the United States to implement newborn screening for SCID, no infants with SCID were missed [63]. Where limited blood is available, for example a post mortem sample, a dried blood spot card can be made and TRECS assayed. TRECS can also be measured from an EDTA blood sample. Although TRECS may be slightly lower in preterm infants, normal TRECs exclude a diagnosis of SCID. Rapid diagnosis of SCID and thymic defects at birth enables optimal treatment and significantly better outcomes [64]. If a patient may have SCID, the Guthrie card can be retrieved and TRECS can be measured on it if the SCID screening was not performed as part of the routine newborn screening program.

### 12.5.3 Immunophenotyping

Laboratory phenotyping of immune cells in the diagnosis of PID focusses on two key aspects. Firstly, quantification of the cells of the immune system and whether these cells express all the proteins required for their function, and secondly, whether the cells can perform their effector functions in *in vitro* assays of immune function.

Immunophenotyping using flow cytometry allows a laboratory to identify whether the patient has the appropriate number of immune cells. An example of this analysis is shown in Fig. 12.3a, showing normal proportions of T cells, B cells and NK cells. If a patient was missing, or had very low numbers of T cells, this would be consistent with a diagnosis of SCID. Whether B cells and/or NK cells were also present/absent, would help to determine the potential underlying genetic defect causing the SCID e.g. a T-B-NK- SCID could be due to ADA or PNP deficiency, whilst a T-B+NK- pattern could be due to defects in gamma chain or JAK3. An example flow cytometry plot of a patient sample with absent T cells and NK cells is shown in Fig. 12.3b. This would be consistent with a diagnosis of gamma chain or JAK3 SCID.

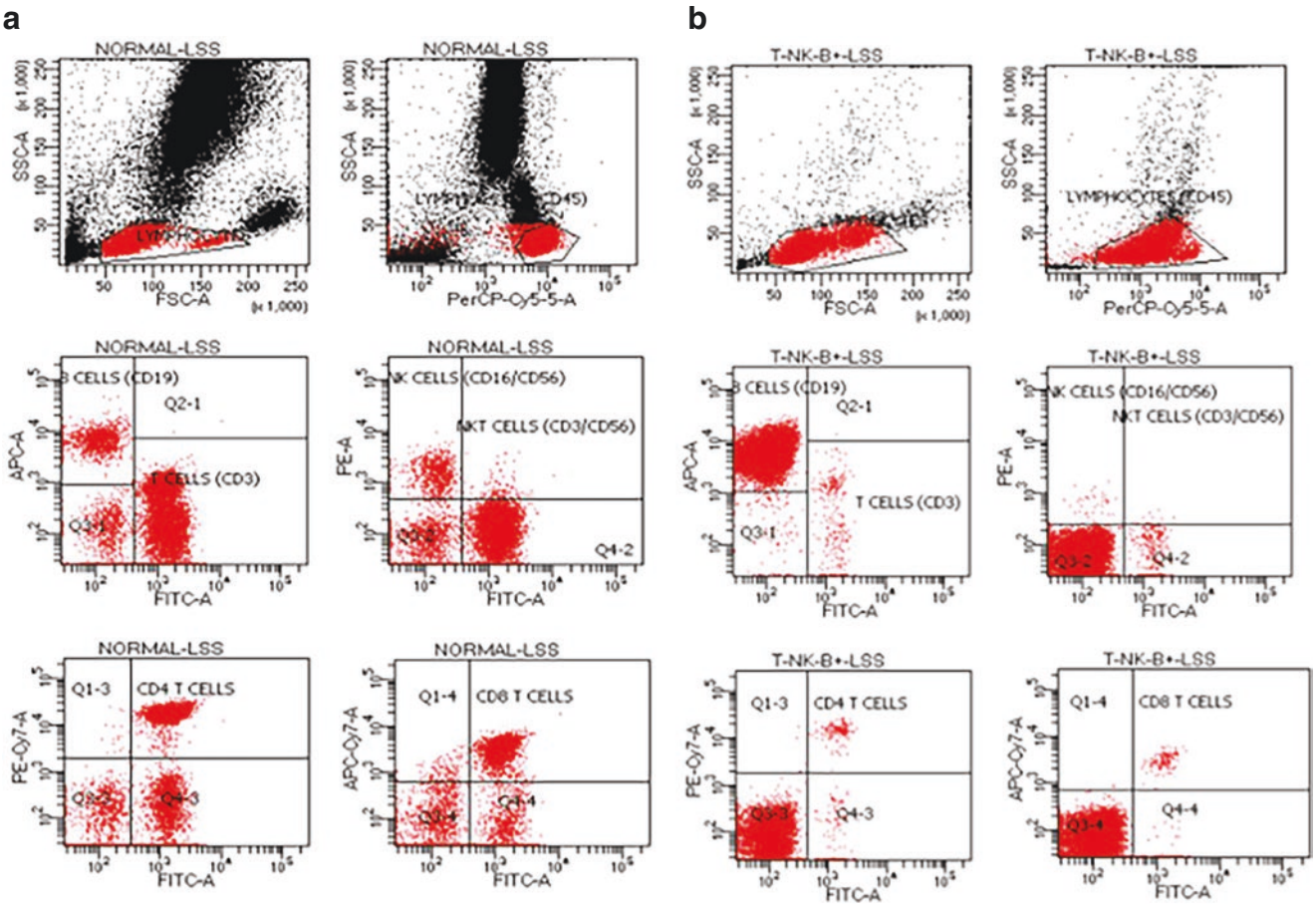


**Fig. 12.3** Immunophenotyping of samples with (**a**) normal proportions of T cells, B cells and NK cells (in this sample 80% T cells, 12% B cells, and 7% NK cells and (**b**) absent T cells and NK cells (in this sample 2% T cells, 98% B cells and 0.2% NK cells respectively). For reference ranges refer to Comans-Bitter et al. 1997 [65]

Flow cytometry can also be applied to determinine whether the cells express the proteins they need to carry out their effector functions. For example, if a patient is identified as having a T-B+NK- immune phenotype consistent with a common gamma chain or JAK3 defect, cells can be stained with a fluorescently labelled antibody against gamma chain. Cells are first 'gated' on lymphocytes, and then gamma chain expression can be examined. Although T cells and NK cells are missing, as the common gamma chain is expressed as part of interleukin receptors on a number of cell types, its expression can still be identified on the remaining B cells. In the case of FLH, the expression of perforin can also be detected by staining a patient sample with a cocktail of fluorescent antibodies to identify NK and CD8 T cells, and perforin to determine whether the patient expresses perforin.

Assays to investigate the in vitro function of immune cells are also of use in the diagnosis of PID. These range from investigating the proliferation of immune cells in response to stimulus as part of the diagnostic work up for SCID, to neutrophil function assays in the diagnosis of disorders such as Chronic Granulomatous Disease (CGD) where there are defects in the killing mechanisms employed by neutrophils.

In all cases, fresh blood samples are required to carry out immunophenotyping investigations. In practice this means ideally within 24–48 h of blood draw for assays involving lymphocytes, and less than 24 h for assay involving neutrophils. Most analyses can be carried out on cord blood, taken either *in utero*, or at birth. Post-mortem samples should be taken peri-mortem for best immunophenotyping results. In assays where a defect in a protein is suspected, a sample from a healthy adult volunteer should be analysed at the same time to ensure the assay is technically valid. Genetic analysis is required to confirm a diagnosis and to identify the precise genetic defect (see below). For deceased infants, it is more important to obtain sufficient material for genetic analysis; however, undertaking immunophenotyping provides important phenotypic information to interpret any genetic variants found.

### 12.5.4 Serum Protein Analysis

As discussed there are a number of serum proteins that contribute to the functioning of the immune system, including

antibodies, complement, cytokines, chemokines, and proteins secreted by innate immune cells such as anti-microbial peptides. Deficiencies in a number of these have been described in patients, but in the majority of cases these are subtle defects that may not manifest in early life, and/or are exceedingly rare. Furthermore, many of these proteins are highly labile and not easily measured in bodily fluids, and where assays exist there are not commonly accepted reference ranges for their measurement. Whilst the presence/absence of these serum proteins is indicative of a PID, it should be noted that *in utero* sampling to measure these proteins is not warranted; the risks from the sampling procedure significantly outweigh the benefits of measuring these proteins.

#### 12.5.4.1 Immunoglobulins

As described, IgG is placentally transferred from the mother to the fetus during gestation, and the neonate will have low levels of IgG in the first 6 months of life until IgG production matures. However, IgA and IgM are not placentally transferred, so any IgA and IgM measured will be produced by the infant. Therefore measuring immunoglobulins is still useful in this case. It is important to note that reference ranges for immunoglobulins are age dependent, so interpretation of individual measurements must be made using the appropriate reference range. Measurement of immunoglobulins is routinely carried out using a technique called nephelometry.

Low/absent IgA and IgM in the face of low normal/low IgG may indicate a defect in the production of immunoglobulin; the IgG present would have been placentally transferred. This lack of immunoglobulin production could be consistent with a primary B cell defect e.g. X-linked agammaglobulinemia (XLA). However, the more likely cause of such a neonatal presentation would be consistent with a diagnosis of SCID, not just a T-B- SCID such as RAG SCID where B cells are low/absent but all forms of SCID as T-cell help is required for immunoglobulin production.

#### 12.5.4.2 Complement

Whilst complement deficiencies are one of the more common PIDs, these do not generally manifest in the fetal/neonatal period. However, severe infection or death as a result of bacterial meningitis should initiate complement investigations.

The complement cascade is a complex system of soluble proteins, and whilst the components C3 and C4 can be measured by nephelometry, measurement of the other components is restricted to a handful of specialist laboratories. If a defect in complement is suspected, then most laboratories can perform a functional assay for complement activity. If normal then a defect in the classical and alternative cascades can be excluded. If abnormal, and technical problems (e.g. in vitro consumption due to poor sample handling) have been excluded, then samples should be referred to a laboratory that carries out testing of individual components. It should be noted that samples need to be frozen within a few hours of blood draw and these tests are rarely suitable from post mortem serum samples unless collected at the time of death.

#### 12.5.4.3 Other Serum Proteins

As highlighted, there is little place for measurement of other serum proteins in the diagnosis of PID in the fetal/neonatal period. One exception to this is the soluble form of the cytokine receptor for IL-2, specifically the alpha chain of this receptor. Whilst soluble IL-2Rα, also known as sCD25, is a non-specific marker of inflammation, it forms part of the laboratory criteria in the diagnostic criteria for HLH along with measurement of serum ferritin [66].

### 12.5.5 Genetic Analysis

Where primary immunodeficiency (PID) is considered, genetic analysis should be undertaken. Where possible this is performed on an EDTA blood sample; however, this can be performed on any available residual tissue. If feasible, growing fibroblasts from a skin biopsy provides an unlimited source of DNA as well as fresh material for functional assays is required. If fresh material is unavailable, DNA may be extracted from frozen tissue or paraffin embedded tissue blocks. If there is a high index of suspicion of a particular disease, targeted genetics is available using Sanger sequencing. However, where one immunophenotype is suggestive of multiple diseases for examples T-B-NK+ SCID is caused by mutations in more than 5 genes, it is more efficient to use next generation sequencing to undertake gene panel analysis (examination of a subset of genes associated with a particular disease), whole exome sequencing or whole genome sequencing. A list of genes causing PID is reviewed every 2 years by the International Union of Immunological Societies with a corresponding classification based on the clinical and laboratory features [67]. Panel App [68] provides a continually curated list of PID gene along with corresponding phenotype, mode of inheritance, reviewers and references. Identification of a genetic cause of immunodeficiency enables targeted treatment including hematopoietic stem cell transplant, thymic transplant, and gene therapy as well as targeted medications such as replacement immunoglobulins for patients with B cell defects. Identification of genetic disease enables family counseling, screening potential carriers and planning for future pregnancies including preimplantation genetic diagnosis.

## 12.6 Summary

As detailed above, recognition of PID and other immune conditions may explain the death of a fetus or neonate and is essential for counseling and family planning for future pregnancies.

## References


1. Tavian M, Coulombel L, Luton D, Clemente HS, Dieterlen-Lievre F, Peault B. Aorta-associated CD34+ hematopoietic cells in the early human embryo. Blood. 1996;87:67–72.
2. Marshall CJ, Sinclair JC, Thrasher AJ, Kinnon C. Bone morphogenetic protein 4 modulates c-Kit expression and differentiation potential in murine embryonic aorta-gonad-mesonephros haematopoiesis in vitro. Br J Haematol. 2007;139:321–30.
3. Krow-Lucal ER, Kim CC, Burt TD, McCune JM. Distinct functional programming of human fetal and adult monocytes. Blood. 2014;123:1897–904.
4. Lawrence SM, Corriden R, Nizet V. The ontogeny of a neutrophil: mechanisms of granulopoiesis and homeostasis. Microbiol Mol Biol Rev. 2018;82:e00057–17.
5. Miller ME, Stiehm ER. Phagocytic, opsonic and immunoglobulin studies in newborns. Calif Med. 1973;119:43–63.
6. Ricklin D, Reis ES, Lambris JD. Complement in disease: a defence system turning offensive. Nat Rev Nephrol. 2016;12:383–401.
7. He YD, Xu BN, Song D, Wang YQ, Yu F, Chen Q, et al. Normal range of complement components during pregnancy: a prospective study. Am J Reprod Immunol. 2020;83:e13202.
8. Regal JF, Gilbert JS, Burwick RM. The complement system and adverse pregnancy outcomes. Mol Immunol. 2015;67:56–70.
9. Hanson LA, Korotkova M. The role of breastfeeding in prevention of neonatal infection. Semin Neonatol. 2002;7:275–81.
10. Haynes BF, Martin ME, Kay HH, Kurtzberg J. Early events in human T cell ontogeny. Phenotypic characterization and immunohistologic localization of T cell precursors in early human fetal tissues. J Exp Med. 1988;168:1061–80.
11. Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. Annu Rev Immunol. 2003;21:139–76.
12. Thilaganathan B, Mansur CA, Morgan G, Nicolaides KH. Fetal T-lymphocyte subpopulations in normal pregnancies. Fetal Diagn Ther. 1992;7:53–61.
13. Debock I, Flamand V. Unbalanced neonatal CD4(+) T-cell immunity. Front Immunol. 2014;5:393.
14. Rechavi E, Lev A, Lee YN, Simon AJ, Yinon Y, Lipitz S, et al. Timely and spatially regulated maturation of B and T cell repertoire during human fetal development. Sci Transl Med. 2015;7:276ra25.
15. Thilaganathan B, Nicolaides KH, Mansur CA, Levinsky RJ, Morgan G. Fetal B lymphocyte subpopulations in normal pregnancies. Fetal Diagn Ther. 1993;8:15–21.
16. Bondada S, Wu H, Robertson DA, Chelvarajan RL. Accessory cell defect in unresponsiveness of neonates and aged to polysaccharide vaccines. Vaccine. 2000;19:557–65.
17. Crotty S. A brief history of T cell help to B cells. Nat Rev Immunol. 2015;15:185–9.
18. Protonotariou E, Malamitsi-Puchner A, Rizos D, Papagianni B, Moira E, Sarandakou A, et al. Age-related differentiations of Th1/Th2 cytokines in newborn infants. Mediators Inflamm. 2004;13:89–92.
19. Nonoyama S, Penix LA, Edwards CP, Lewis DB, Ito S, Aruffo A, et al. Diminished expression of CD40 ligand by activated neonatal T cells. J Clin Invest. 1995;95:66–75.
20. Gilmour KC, Walshe D, Heath S, Monaghan G, Loughlin S, Lester T, et al. Immunological and genetic analysis of 65 patients with a clinical suspicion of X linked hyper-IgM. Mol Pathol. 2003;56:256–62.
21. GenScript. IgM antibody 2020. Available from: https://www.genscript.com/IgM-antibody.html
22. Conway SP, Dear PR, Smith I. Immunoglobulin profile of the preterm baby. Arch Dis Child. 1985;60:208–12.
23. Palmeira P, Quinello C, Silveira-Lessa AL, Zago CA, Carneiro-Sampaio M. IgG placental transfer in healthy and pathological pregnancies. Clin Dev Immunol. 2012;2012:985646.
24. Ravivarma KR, Babar ST, Master J, Bapat JP, Baxi AJ. Immunoglobulins in newborns: differential study of premature and full term infants. J Postgrad Med. 1979;25:97–101.
25. Cutbush M, Mollison PL. Relation between characteristics of blood-group antibodies in vitro and associated patterns of redcell destruction in vivo. Br J Haematol. 1958;4:115–37.
26. Williams RT. Acquired dysgammaglobulinaemia in a young man. Clin Exp Immunol. 1966;1:223–31.
27. Iijima S. Sporadic isolated congenital asplenia with fulminant pneumococcal meningitis: a case report and updated literature review. BMC Infect Dis. 2017;17:777.
28. Vellguth S, von Gaudecker B, Muller-Hermelink HK. The development of the human spleen. Ultrastructural studies in fetuses from the 14th to 24th week of gestation. Cell Tissue Res. 1985;242:579–92.
29. Boyaka PN, Wright PF, Marinaro M, Kiyono H, Johnson JE, Gonzales RA, et al. Human nasopharyngeal-associated lymphoreticular tissues. Functional analysis of subepithelial and intraepithelial B and T cells from adenoids and tonsils. Am J Pathol. 2000;157:2023–35.
30. Good RA, Zak SJ, Condie RM, Bridges RA. Clinical investigation of patients with agammaglobulinemia and hypogammaglobulinemia. Pediatr Clin North Am. 1960;7:397–433.
31. Gaspar HB, Conley ME. Early B cell defects. Clin Exp Immunol. 2000;119:383–9.
32. Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Seidel H, et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. J Med Genet. 1997;34:798–804.
33. Tezenas Du Montcel S, Mendizabai H, Ayme S, Levy A, Philip N. Prevalence of 22q11 microdeletion. J Med Genet. 1996;33:719.
34. Carlson C, Sirotkin H, Pandita R, Goldberg R, McKie J, Wadey R, et al. Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. Am J Hum Genet. 1997;61:620–9.
35. Yagi H, Furutani Y, Hamada H, Sasaki T, Asakawa S, Minoshima S, et al. Role of TBX1 in human del22q11.2 syndrome. Lancet. 2003;362:1366–73.
36. Sullivan KE. Chromosome 22q11.2 deletion syndrome and DiGeorge syndrome. Immunol Rev. 2019;287:186–201.
37. Van Esch H, Groenen P, Fryns JP, Van de Ven W, Devriendt K. The phenotypic spectrum of the 10p deletion syndrome versus the classical DiGeorge syndrome. Genet Couns. 1999;10:59–65.
38. Digilio MC, Marino B, Formigari R, Giannotti A. Maternal diabetes causing DiGeorge anomaly and renal agenesis. Am J Med Genet. 1995;55:513–4.
39. Cavdar AO. DiGeorge's syndrome and fetal alcohol syndrome. Am J Dis Child. 1983;137:806–7.
40. Coberly S, Lammer E, Alashari M. Retinoic acid embryopathy: case report and review of literature. Pediatr Pathol Lab Med. 1996;16:823–36.
41. Gholam C, Grigoriadou S, Gilmour KC, Gaspar HB. Familial haemophagocytic lymphohistiocytosis: advances in the genetic basis, diagnosis and management. Clin Exp Immunol. 2011;163:271–83.
42. Davies EG. Immunodeficiency in DiGeorge Syndrome and options for treating cases with complete athymia. Front Immunol. 2013;4:322.

43. Issekutz KA, Graham JM Jr, Prasad C, Smith IM, Blake KD. An epidemiological analysis of CHARGE syndrome: preliminary results from a Canadian study. Am J Med Genet A. 2005;133A:309–17.
44. Janssen N, Bergman JE, Swertz MA, Tranebjaerg L, Lodahl M, Schoots J, et al. Mutation update on the CHD7 gene involved in CHARGE syndrome. Hum Mutat. 2012;33:1149–60.
45. Jyonouchi S, McDonald-McGinn DM, Bale S, Zackai EH, Sullivan KE. CHARGE (coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, ear anomalies/deafness) syndrome and chromosome 22q11.2 deletion syndrome: a comparison of immunologic and nonimmunologic phenotypic features. Pediatrics. 2009;123:e871–7.
46. Wong MT, Scholvinck EH, Lambeck AJ, van Ravenswaaij-Arts CM. CHARGE syndrome: a review of the immunological aspects. Eur J Hum Genet. 2015;23:1451–9.
47. Buckley RH, Schiff RI, Schiff SE, Markert ML, Williams LW, Harville TO, et al. Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. J Pediatr. 1997;130:378–87.
48. Heeg M, Ammann S, Klemann C, Panning M, Falcone V, Hengel H, et al. Is an infectious trigger always required for primary hemophagocytic lymphohistiocytosis? Lessons from in utero and neonatal disease. Pediatr Blood Cancer. 2018;65:e27344.
49. Suzuki N, Morimoto A, Ohga S, Kudo K, Ishida Y, Ishii E, et al. Characteristics of hemophagocytic lymphohistiocytosis in neonates: a nationwide survey in Japan. J Pediatr. 2009;155:235–8.
50. Iwatani S, Uemura K, Mizobuchi M, Yoshimoto S, Kawasaki K, Kosaka Y, et al. Familial hemophagocytic lymphohistiocytosis presenting as hydrops fetalis. AJP Rep. 2015;5:e22–4.
51. Sillers L, Van Slambrouck C, Lapping-Carr G. Neonatal thrombocytopenia: etiology and diagnosis. Pediatr Ann. 2015;44:e175–80.
52. Louie RJ, Tan QK, Gilner JB, Rogers RC, Younge N, Wechsler SB, et al. Novel pathogenic variants in FOXP3 in fetuses with echogenic bowel and skin desquamation identified by ultrasound. Am J Med Genet A. 2017;173:1219–25.
53. Makitie O, Vakkilainen S. Cartilage-hair hypoplasia - anauxetic dysplasia spectrum disorders. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, LJH B, Stephens K, et al., editors. GeneReviews((R)). Seattle, WA: University of Washington; 2018.
54. Freeman AF, Holland SM. Clinical manifestations of hyper IgE syndromes. Dis Markers. 2010;29:123–30.
55. Marques AH, O'Connor TG, Roth C, Susser E, Bjorke-Monsen AL. The influence of maternal prenatal and early childhood nutrition and maternal prenatal stress on offspring immune system development and neurodevelopmental disorders. Front Neurosci. 2013;7:120.
56. Lenti E, Farinello D, Yokoyama KK, Penkov D, Castagnaro L, Lavorgna G, et al. Transcription factor TLX1 controls retinoic acid signaling to ensure spleen development. J Clin Invest. 2016;126:2452–64.
57. Brito-Zeron P, Izmirly PM, Ramos-Casals M, Buyon JP, Khamashta MA. The clinical spectrum of autoimmune congenital heart block. Nat Rev Rheumatol. 2015;11:301–12.
58. Mannisto T, Mendola P, Reddy U, Laughon SK. Neonatal outcomes and birth weight in pregnancies complicated by maternal thyroid disease. Am J Epidemiol. 2013;178:731–40.
59. Cameo T, Gumer LB, Williams KM, Gomez J, McMahon DJ, Oberfield SE. A retrospective review of newborn screening for congenital hypothyroidism and newborn thyroid disease at a major medical center. Clin Pediatr (Phila). 2013;52:1054–8.
60. Bucci I, Giuliani C, Napolitano G. Thyroid-stimulating hormone receptor antibodies in pregnancy: clinical relevance. Front Endocrinol (Lausanne). 2017;8:137.
61. Papazian O. Transient neonatal myasthenia gravis. J Child Neurol. 1992;7:135–41.
62. Zhao Y, Dai ZP, Lv P, Gao XM. Phenotypic and functional analysis of human T lymphocytes in early second- and third-trimester fetuses. Clin Exp Immunol. 2002;129:302–8.
63. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA. 2014;312:729–38.
64. Brown L, Xu-Bayford J, Allwood Z, Slatter M, Cant A, Davies EG, et al. Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening. Blood. 2011;117:3243–6.
65. Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr. 1997;130:388–93.
66. Bergsten E, Horne A, Arico M, Astigarraga I, Egeler RM, Filipovich AH, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative HLH-2004 study. Blood. 2017;130:2728–38.
67. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, et al. The 2017 IUIS phenotypic classification for primary immunodeficiencies. J Clin Immunol. 2018;38:129–43.
68. PanelApp. Primary immunodeficiency (Version 2.175) 2019. Available from: https://panelapp.genomicsengland.co.uk/panels/398/