twin-twin transfusion. Acute twin-twin transfusion generally results in twins of similar size but with Hb concentrations that vary by more than 5 g/dL [75, 76]. In chronic twin-twin transfusion, the donor twin becomes progressively anemic and growth retarded, whereas the recipient twin becomes polycythemic, macrosomic, and sometimes hypertensive. Both can develop hydrops fetalis; the donor twin becomes hydropic from profound anemia, and the recipient twin from congestive heart failure and hypervolemia. The donor twin often has low amniotic fluid volumes, whereas the recipient twin has increased amniotic fluid, due to significant differences in blood volume, renal blood flow, and urine output.

Chronic twin-twin transfusion can be diagnosed by serial prenatal ultrasound measuring cardiomegaly, discordant amniotic fluid production, and fetal growth discrepancy of >20%. Percutaneous umbilical blood sampling can determine if Hb concentration differences of greater than 5 gm/dL exist. After birth, the donor twin may require transfusions and can have neutropenia, hydrops from severe anemia, growth retardation, congestive heart failure, and hypoglycemia. The recipient twin is often the sicker of the two, with problems including hypertrophic cardiomyopathy, congestive heart failure, polycythemia, hyperviscosity, respiratory difficulties, hypocalcemia, and hypoglycemia. Neurologic evaluation and imaging are imperative because the risk of antenatally acquired neurologic cerebral lesions is 20–30% in both twins. The incidence of neurologic morbidity following the intrauterine death of one of the fetuses averages 20–25%. Morbidities include multiple cerebral infarctions, hypoperfusion syndromes from hypotension, and periventricular leukomalacia. Long-term neurologic follow-up is indicated for all survivors of twin-twin transfusion [75, 76].

Prenatal treatment for twin-twin transfusion consists of close monitoring and reduction amniocenteses to decrease uterine stretch and prolong the pregnancy. Selective feticide of the hydropic twin has been advocated by some and has resulted in the survival of the healthier twin in some studies [77]. Treatment in utero has occurred during some pregnancies using laser ablation of bridging vessels, resulting in improved survival rates up to around 50%, with approximately 70% of the pregnancies having at least one survivor [78–80]. However, the survival rate without morbidity in the surviving twin is approximately 50%. Supski et al performed a meta analysis of 140 cases to correlate types of treatment with outcome [81]. They found no differences in outcome between amnioreduction, fetoscopy, septostomy, or close observation.

103.6.2 Perinatal Hemorrhage

Perinatal blood loss to the fetus can occur with various obstetric complications, such as placenta previa, placental abruption, incision or tearing of the placenta during cesarean section, and cord evulsion. When a fetus undergoes significant blood loss back into the placenta the term fetoplacental hemorrhage is used. Placental anomalies such as a multilobed placenta and placental chorioangiomas can be a source of perinatal bleeding [82].

Placental abruption occurs in 3–6 per 1000 live births. Risk factors of placental abruption include prolonged rupture of the membranes, severe fetal growth restriction, chorioamnionitis, hypertension, maternal diabetes, cigarette smoking, and advanced maternal age [83]. The incidence of abruption increases with lower gestational age. Neonatal mortality rates from abruption range from 0.8–2.0 per thousand births, or 15–20% of the deliveries in which significant abruption occurs.

Women with a history of a previous cesarean birth and increased parity are at increased risk of placenta previa [84], a condition where part or all of the placenta overlies the cervical os. Cigarette smoking is associated with a 2.6- to 4.4-fold increased risk of placenta previa [85]. Prenatal diagnosis of vasa previa (anomalous vessels overlying the internal os of the cervix) can be made with transvaginal color Doppler, and should be suspected in cases of antepartum or intrapartum hemorrhage. Although uncommon (1 in 3000 deliveries), the perinatal death rate is high, ranging from 33–100% when this condition is undetected before delivery [86].

Neonates delivered after placental abruption or after placenta previa can be anemic but they can also have signs of hypoxia and ischemia. The majority of blood lost in an abruption or previa is maternal blood, but the neonate can have some degree of anemia as well. Therefore, when perinatal blood loss is recognized or suspected, the neonate's Hb should be measured at birth and again 12 hours or so later. A Kleihauer Betke stain can be performed on maternal blood to determine if fetal hemorrhage can be documented. Monitoring bleeding mothers with ultrasound might detect placental abnormalities.

Cord rupture due to traction on a shortened or abnormal umbilical cord usually occurs on the fetal side. Cord aneurysms, varices, and cysts can all lead to a weakened cord. Cord infections (funisitis) can also weaken the cord and increase the risk of rupture. Infants born precipitously may be at increased risk for hemorrhage due to a ruptured cord.

Cord hematomas occur infrequently (1 in 5000–6000 deliveries) and can be a cause of fetal blood loss and perinatal mortality. Intrauterine death can occur due to compression of the umbilical vessels by a cord hematoma. Cord hematomas can result from trauma from percutaneous umbilical blood sampling. Hematomas of the cord can be diagnosed in utero by ultrasound [86, 87].

Subamniotic hematomas can occur when chorionic vessels rupture near the cord insertion. Most subamniotic hematomas are the result of traction on a normal or shortened umbilical cord and are not noted until after delivery.

Velamentous insertion of the umbilical cord occurs when the umbilical cord enters the membranes distant from the placenta. This is present in 0.5–2.0% of pregnancies [88]. Blood vessels left unprotected by Wharton jelly are more likely to tear. Rupture of anomalous vessels in the absence of traction or trauma can occur even if the cord itself attaches

centrally or paracentrally. Fetal mortality remains very high in this condition, often because detection by routine ultrasound is rare [89].

103.6.3 Postnatal Hemorrhage

Loss of fetal blood into the placenta can occur during delivery. In fact, a net shift of blood from the fetus into the placenta is a rather common cause of low-grade neonatal anemia. At term, the fetal-placental-umbilical cord unit contains about 120 mL of blood per kg body weight. After delivery, but before the umbilical cord is severed, blood in this unit can flow predominantly toward or away from the neonate. A fetoplacental hemorrhage can occur when the neonate is held significantly higher than the placenta after birth. Also, neonates can lose up to 20% of their blood volume when born with a tight nuchal cord, which allows blood to be pumped through umbilical arteries toward the placenta, while constricting flow back from the placenta to the baby, through the umbilical vein, which is more easily constricted due to its thin wall.

As shown in Fig. 103.5, blood loss can occur into the subgaleal space before or after birth. This is seen most commonly with difficult deliveries requiring vacuum or forceps assistance. Subgaleal hemorrhages are potentially life-threatening and must be recognized as early as possible to prevent significant morbidity or mortality. The hemorrhage occurs when bridging veins are torn, allowing blood to accumulate in the large potential space between the galea aponeurotica and the periosteum of the skull. The subgaleal space extends from the orbital ridge to the base of the skull and can accommodate a volume equivalent to a neonate's entire blood volume [90, 91].

Subgaleal hematomas can form because of risk factors such as coagulopathy or asphyxia, but vacuum extraction itself is a risk factor for their development. The diagnosis should be considered in the presence of a ballotable fluid collection in dependent regions of the infant's head, coupled with signs of hypovolemia [92]. Treatment requires restoration of blood volume and control of bleeding. Exsanguination due to subgaleal hemorrhage has been reported. A suggested way to estimate the volume of blood lost is by following head circumference; 40 mL of blood has been lost for every 1 cm increase in head circumference that occurs [93]. The duration of vacuum application is thought to be the best predictor of scalp injury, followed by duration of second stage of labor and paramedian cup placement. Of those with reported subgaleal hemorrhages, 80–90% had some history of vacuum or instrument-assisted delivery [92, 93]. Limiting the frequency and duration of vacuum assistance in high-risk infants might decrease the incidence of subgaleal hematomas.

Anemia appearing after the first 24 hours of life in a nonjaundiced infant can be a sign of hemorrhage. Hemorrhages

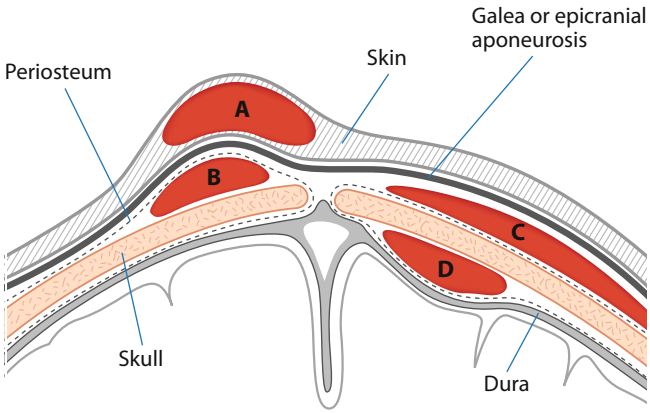

Fig. 103.5 A schematic drawing of the scalp and skull of a newborn infant, illustrating four distinct anatomic spaces where fluid or blood might collect during or after delivery. **A** caput hemorrhage, **B** cephalhematoma, **C** subgaleal hemorrhage, **D** extradural hemorrhage

can be visible, such as a cephalohematoma, or occult. Breech deliveries can be associated with renal, adrenal, or splenic hemorrhage into the retroperitoneal space. Delivery of macrosomic infants, such as infants born to diabetic mothers, can result in hemorrhage. Infants with overwhelming sepsis can bleed into soft tissue and organs.

In addition to causing anemia, adrenal hemorrhage can result in circulatory collapse due to the loss of organ function. The incidence of adrenal hemorrhage is 1.7 per 1000 births [94]. Adrenal hemorrhage can also affect surrounding organs. Intestinal obstruction and kidney dysfunction have been reported in infants with adrenal hemorrhage [95]. The diagnosis can be made using ultrasonography, during which calcifications or cystic masses are noted. Adrenal hemorrhage can be distinguished from renal vein thrombosis (RVT) by ultrasound, in that RVT generally results in a solid mass. Occasionally, both entities coexist in the same patient.

The liver of a neonate is prone to iatrogenic rupture, resulting in high morbidity and mortality [96]. Neonates with this problem can appear asymptomatic until the liver ruptures and hemoperitoneum occurs. This problem can occur in both term and preterm infants [97] and has been associated with chest compressions during cardiopulmonary resuscitation. Surgical intervention has been reported to save some infants but the mortality is very high [97].

Splenic rupture can result from birth trauma or as a result of distention caused by extramedullary hematopoiesis, such as that seen in erythroblastosis fetalis. Abdominal distension and discoloration, scrotal swelling, and pallor are clinical signs of splenic rupture; these can also be seen with adrenal or hepatic hemorrhage [96–98].

More rare causes of hemorrhage in the newborn period include hemangiomas of the gastrointestinal tract [99], vascular malformations of the skin, and hemorrhage into soft tumors, such as giant sacrococcygeal teratomas. Occult intraabdominal hemorrhage can occur with fetal ovarian cysts, which are

usually benign and resolve spontaneously. One case of fetal anemia was diagnosed by a spontaneous hemorrhage into a fetal ovarian cyst and was managed by intrauterine blood transfusions [100].

103.7 Fetal and Neonatal Anemia Due to Congenital Infection

Neonatal bacterial sepsis can cause anemia on the basis of hemolysis, DIC, and hemorrhage. Some microorganisms responsible for neonatal sepsis produce hemolytic endotoxins that result in accelerated erythrocyte destruction, often associated with a microangiopathic process [101].

Congenital viral infections can also cause hemolytic anemia. Congenital syphilis can present with hemolytic anemia. Initial maternal screening for syphilis can be negative despite overwhelming infection, a condition termed the prozone effect [101], which occurs when a higher than optimal amount of antibody in the tested sera prevents the flocculation reaction seen in positive reagin test. In cases of nonimmune hydrops, nontreponemal testing should be repeated using serum dilutions to prevent a missed diagnosis of syphilis in women with negative syphilis serologic results.

Fetal and neonatal infection with parvovirus B19 can cause severe anemia, hydrops, and fetal demise [102]. This is generally a hypoplastic anemia, but hemolysis can occur as well. The virus replicates in erythroid progenitor cells and results in red cell aplasia. In utero transfusions for hydropic fetuses can be successful. Intrauterine fetal infusion of B19 IgG-rich high titer gamma globulin has been reported to be successful [103].

Other fetal infections associated with neonatal anemia include malaria and HIV. Congenital malaria is seen rarely in the USA, generally in large cities where imported cases of malaria are increasing. In certain African countries, congenital malaria has been reported in up to 20% of neonates [104]. Congenital HIV infection in a neonate is generally asymptomatic. However infants born to mothers on zidovudine can have a hypoplastic anemia due to suppressive effects of the drug on fetal erythropoiesis [105].

103.8 Anemia of Prematurity and Other Hypoproliferative Disorders

Impaired erythrocyte production can occur in a fetus or neonate for a variety of reasons. Lack of an appropriate or sufficient marrow environment (as seen in osteopetrosis), lack of specific substrates or their carriers (e.g., iron, folate, vitamin B12, or transcobalamin II deficiency) and lack of specific growth factors (e.g., decreased Epo production or abnormalities in Epo receptors) can be causative.

103.8.1 Anemia of Prematurity

Infants delivered before 32 completed weeks gestation typically develop a transient and unique anemia knows as the anemia of prematurity. During the first week or two after birth, while in an intensive care unit, anemia secondary to phlebotomy loss is common. However, after this period has passed, a second anemia is sometimes seen; characterized as a normocytic, normochromic, hyporegenerative anemia, with serum Epo concentrations significantly below those found in adults with similar degrees of anemia [106]. This anemia is not responsive to the administration of iron, folate, or vitamin E. Some infants with the anemia of prematurity are asymptomatic, whereas others have clear signs of anemia that are alleviated by erythrocyte transfusion. These signs include tachycardia, rapid tiring with nipple feedings, poor weight gain, increased requirements for supplemental oxygen, episodes of apnea and bradycardia, and elevated serum lactate concentrations.

The reason preterm infants do not significantly increase serum Epo concentration during this anemia is not known. Indeed, it is unclear whether production of Epo does in fact increase, yet the serum concentration does not. Certainly their erythroid progenitors are sensitive to Epo [107, 108], and concentrations of other erythropoietic growth factors appear to be normal [109].

The molecular and cellular mechanisms responsible for the anemia of prematurity remain undefined. Some explanations include the transition from fetal to adult Hb, shortened erythrocyte survival, and hemodilution associated with a rapidly increasing body mass [110]. It is unknown whether preterm infants rely on Epo produced by the liver (the source of Epo in utero), or that produced by the kidney, or a combination of the two. Regardless of the mechanism responsible for the anemia of prematurity, exogenous Epo administered to preterm infants accelerates effective erythropoiesis [111]. A meta-analysis of studies evaluating the use of "late" Epo administration to prevent and treat the anemia of prematurity reveals a positive effect on decreasing transfusion requirements in preterm infants [112]. In addition, beneficial neurodevelopmental effects of recombinant Epo administration have been reported in preterm infants [113–116].

Pharmacokinetic studies of darbepoetin, the long-acting erythropoietic stimulator, have been conducted among neonates with the anemia of prematurity, with the speculation that less frequent dosing and cost savings might render Darbepoetin a more attractive alternative than recombinant Epo

Table 103.5 Terminal half-life of darbepoetin among adults, children, and neonates after subcutaneous or intravenous dosing

	After subcutaneous dosing	After intravenous dosing
Adults	49 hours	25 hours
Children	43 hours	22 hours
Neonates	22 hours	10 hours

for treating the anemia of prematurity [117–119]. Following subcutaneous and intravenous dosing, Darbepoetin has a considerably shorter terminal half-life in neonates than in adults (Table 103.5). Intravenous dosing appears to be as effective as subcutaneous dosing.

103.8.2 Other Hypoproliferative Anemias and Associated Syndromes

During the neonatal period hypoproliferative anemias are rare, with the exception of the anemia of prematurity, which is common (Table 103.6). Diamond-Blackfan syndrome can be diagnosed at birth but usually is not recognized until after 2–3 months of age. At least 10–25% of infants with Diamond-Blackfan syndrome have anemia at birth [120, 121], and severe anemia with hydrops has been reported. Aase syndrome, another congenital hypoplastic anemia syndrome involving skeletal anomalies [122], is sometimes classified as a variant of Diamond-Blackfan syndrome. Congenital dyserythropoietic anemia is a rare disorder marked by ineffective erythropoiesis, megaloblastic anemia, and characteristic abnormalities of the nuclear membrane and cytoplasm seen on electron microscopy. Fanconi anemia almost never manifests during the neonatal period. This autosomal-recessive disorder is characterized by marrow failure and congenital anomalies, including abnormalities in skin pigmentation, gastrointestinal anomalies, renal anomalies, and upper limb anomalies [123].

Osteopetrosis involves osteoclast dysfunction, resulting in a decreased marrow space [124, 125]. Developmental delay, ocular involvement, and neurodegenerative findings occur in these patients in association with hypoplastic anemia. Patients are generally treated with stem cell transplantation, but they are particularly susceptible to post-transplantation complications after myeloablation, and reduced-intensity conditioning programs may be helpful.

Pearson syndrome is a congenital hyporegenerative anemia that can progress to pancytopenia, and additionally affects the exocrine pancreas, liver, and kidneys [126]. These patients can present during the neonatal period, but typically do so later in infancy. Features include failure to thrive and cytopenia. The marrow examination shows characteristic vacuoles within erythroid and myeloid precursors, hemosiderosis, and ringed sideroblasts. The syndrome is caused by a loss of large segments of mitochondrial DNA [127, 128].

Table 103.6 Syndromes associated with congenital anemia

Syndrome	Phenotypic features	Genotypic features
Adenosine deaminase deficiency	Autoimmune hemolytic anemia, reduced erythrocyte adenosine deaminase activity	AR, 20q13.11
Congenital dyserythropoietic anemias	Type I (rare): megaloblastoid erythroid hyperplasia and nuclear chromatin bridges between nuclei; type II (most common): "hereditary erythroblastic multinuclearity, positive acidified serum (HEMPAS) test, increased lysis to anti-i; type III: erythroblastic multinuclearity ("gigantoblasts"), macrocytosis	Type I: 15q15.1-q15.3; type II: 20q11.2; type III: 15q21
Diamond-Blackfan syndrome	Steroid-responsive hypoplastic anemia, often macrocytic after 5 months of age	AR; sporadic mutations and AD inheritance described; 19q13.2, 8p23.3-p22
Dyskeratosis congenita	Hypoproliferative anemia usually presenting between 5–15 yr of age	X-linked recessive, locus on Xq28; some cases with AD inheritance
Fanconi pancytopenia	Steroid-responsive hypoplastic anemia, reticulocytopenia, some macrocytic RBCs, shortened RBC lifespan. Cells are hypersensitive to DNA cross-linking agents	AR, multiple genes: complementation; group A: 16q24.3; B:; C: 9q22.3; D2: 3p25.3; E: 6p22-p21; F: 11p15; G: 9p13
Osler hemorrhagic telangiectasia syndrome	Hemorrhagic anemia	AD, 9q34.1
Osteopetrosis	Hypoplastic anemia from marrow compression; extramedullary erythropoiesis.	AR: 16p13, 11q13.4-q13.5; AD: 1p21; lethal: reduced osteoclasts
Pearson syndrome	Hypoplastic sideroblastic anemia, marrow cell vacuolization	Pleioplasmatic rearrangement of mitochondrial DNA; X-linked or AR
Peutz-Jeghers syndrome	Iron deficiency anemia from chronic blood loss	AD, 19p13.3
X-linked α-thalassemia/ mental retardation (ATR-X and ATR-16) syndromes	ATR-X: hypochromic, microcytic anemia; mild form of hemoglobin H disease ATR-16: more significant hemoglobin H disease and anemia are present	ATR-X: X-linked recessive, Xq13.3; ATR-16: 16p13.3, deletions of α-globin locus

AD autosomal dominant, *AR* autosomal recessive, *RBC* red blood cell.

103.9 Considerations Regarding Erythrocyte Transfusion in the Neonatal Period

Best practices in neonatal transfusion medicine are largely undefined. A very basic issue that remains unsettled is at what level to keep the Hb concentration during the NICU stay. Specifically, it is not clear whether to keep a NICU patient's Hb as high as it would be in utero (often requiring multiple transfusions to do so) or whether to permit the Hb to fall to considerably lower values, attempting to avoid or minimize transfusions. Attempts have been made to define the best Hb range for NICU patients, but study findings are discordant.

In a single-centered study, Bell et al randomized 100 neonates with birth weights <1300 g (average birth weight 956 grams) to maintain a hematocrit in a higher range versus a lower range. Those kept in the lower range received fewer transfusions (average of 2 additional transfusions per patient), but were more likely to have intraparenchymal brain hemorrhage or periventircular leukomalacia [129]. In contrast, the PINT study (Premature Infants in Need of Transfusion), a larger multicentered study involving 451 extremely low birth weight neonates (average birth weight 770 grams), concluded that neonates randomized to the lower hematocrit range had fewer transfusions but similar neurodevelopmental outcomes [130].

Various neonatal transfusion guidelines have been used over the last 2 decades, and research is ongoing to determine the optimal strategy for administering red cell transfusions to preterm and term neonates. One strategy developed by the Canadian Paediatric Society in 2002 is shown in Table 103.7. When considering a transfusion in a preterm infant with a low hematocrit which is not due to acute hemorrhage, it should be determined if the infant needs an immediate increase in oxygen to tissues. If so, then treatment consists of a transfusion of packed red cells. If there is no evidence that an immediate increase in oxygen delivery is necessary, then treatment with red cell growth factors and appropriate substrates might be considered. As the process of stimulating erythropoiesis requires at least a week to significantly impact the reticulocyte count, and may not appreciably increase the hemoglobin concentration during that time, the infant should continue to be observed for signs consistent with anemia.

A method of reducing erythrocyte transfusions, among a subset of preterm neonatal patients, is to begin the administration of recombinant Epo to those with low hematocrits after the first three weeks of life [110–113]. Recombinant Epo certainly stimulates erythropoiesis in such patients, although its combination with additional folate, iron, vitamin E, and vitamin B12 may be superior to recombinant Epo alone [132].

Haiden and colleagues achieved significantly greater success in preterm infants <800 grams (38% of infants not transfused) when vitamin B12 at a dose of 21 mg/kg/week SC was added to a regimen of Epo, iron, vitamin E and folate [132]. When combined with limited phlebotomy losses, this therapy shows great promise in ELBW infants.

Another method of reducing erythrocyte transfusions to ill neonates is to delay clamping the umbilical cord. Even a delay of 30 seconds can result in improved iron status [133], fewer transfusions [130], and perhaps superior neurodevelopmental outcomes [134-137].

Yet another method of reducing or postponing early erythrocyte transfusions among extremely low birth weight neonates is to draw the blood for the initial laboratory tests from the placenta not from the neonate. The initial phlebotomy of an extremely low birth weight neonate, on admission to the NICU, can include a blood culture, CBC, type and cross-match, metabolic screen, blood gas, electrolytes, and glucose. Sometimes other studies such as coagulation tests are also drawn at or shortly following NICU admission. The total blood volume needed for these base-line NICU laboratory studies can be 4–5 mL or more. In a 400–500 gram neonate this can exceed 10% of the total blood volume. Early transfusions in the NICU can also be reduced by careful attention to phlebotomy volumes during the first days following delivery. Transfusions given during the first week or two are principally to replace phlebotomy losses for laboratory tests. Employing laboratory methods that minimize blood loss will reduce early transfusions. Such methods include point of care monitors, point of care analyzers, and a concerted effort to use the smallest amount of blood possible for the needed laboratory studies [137–138].

Table 103.7 Canadian Paediatric Society recommendations for RBC transfusions in newborn infants

- Acute blood loss resulting in hypovolemic shock
- Hemoglobin between 10 and 12 g/dL or hematocrit between 30 and 35% in extreme illness conditions for which RBC transfusion may improve oxygen delivery to vital organs
- Hemoglobin between 6 and 10 g/dL or hematocrit between 20% and 30%, in severely ill neonates and/or on mechanical ventilation with compromised oxygen delivery
- Hemoglobin below 6 g/dL or hematocrit below 20% with absolute reticulocyte count 100–150 × 10^3/μL or less, suggesting low plasma concentration of erythropoietin, with the presence of the following clinical signs: poor weight gain, heart rate >180 beats/minute, respiratory distress and increased oxygen needs, and lethargy

Modified from [131].

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Fetal and Neonatal Hydrops

Gennaro Vetrano and Mario De Curtis

104.1 Introduction

Hydrops fetalis (i.e., fetal hydrops) (HF) is a serious condition defined as an abnormal accumulation of fluid in two or more fetal compartments. It presents as ascites, pleural effusion, pericardial effusion and skin edema. In some patients, it may also be associated with polyhydramnios and placental edema. In 1943, Potter was the first to distinguish non-immune hydrops fetalis (NIHF) from immune hydrops [1].

104.2 Epidemiology

Previously, hemolytic disease due to Rh incompatibility was the main cause of both fetal and neonatal immune hydrops. These days approximately 90% of cases of hydrops fetalis are due to non-immune disease, with the number of liveborn ranging from 1:1500 to 1:3800 [2, 3]. Hydrops fetalis is much more common in Southeast Asia; in Thailand the expected frequency of non-immune hydrops fetalis due to homozygous alpha-thalassemia or Bart hydrops ranges from one every 500 to one every 1500 pregnancies [4, 5]. Although the availability of ultrasound technology has greatly improved antenatal diagnosis of HF, perinatal mortality (PNM) remains high.

104.3 Pathogenesis

The basic mechanism for the formation of HF is an imbalance between interstitial fluid production and lymphatic return. Fluid accumulation in the fetus can be due to (a) heart failure, (b) anemia, (c) obstructed lymphatic flow, or (d) decreased plasma osmotic pressure. The fetus is particularly susceptible to interstitial fluid accumulation because of its greater capillary permeability, compliant interstitial compartments, and liability for raised venous pressure because of impaired lymphatic return [5, 6]. Clinical and animal studies have shown that elevation of central venous pressure (CVP) has a pivotal role in the development of fetal hydrops [7]. Increased CVP causes edema and effusions by increasing capillary hydrostatic pressure and decreasing lymphatic return [8]. Albumin is the main oncotically active plasma protein and when its hepatic synthesis is impaired, transcapillary fluid movements increase [5, 6]. Hypoproteinemia and hypoalbuminemia are common in human hydrops. However, studies in humans and animals have shown that hypoalbuminemia is unlikely to trigger this condition [9].

104.4 Etiology

The possible etiologies of HF are still being unrevealed, as previously unknown causes are reported. However, despite extensive pre- and postnatal investigations, no definite cause can be determined in 33% of cases [10]. HF is an end-stage process and a non-specific finding that is associated with a range of abnormalities. Its causes can be divided into 6 broad categories: hematological disorders, cardiovascular and infectious conditions, genetic abnormalities, tumors, and idiopathic.

Table 104.1 summarizes the causes of fetal hydrops.

104.5 Diagnosis

A pregnant woman with polydramnios, severe anemia, toxemia or isoimmune disease should undergo further investigation.

The antenatal diagnosis of HF is made by the ultrasound finding of fluid accumulation in the fetus or placenta. Specifically, excess serous fluid should be identified in at least one

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Table 104.1 Causes of hydrops fetalis

Hematological
- Isoimmunization (immune hydrops) (hemolytic disease of the newborn, erythroblastosis)
  - Rh (most commonly D; also C, c, E, e)
  - Kell
  - ABO
  - Others
- Other hemolytic disorders
  - Glucose phosphate isomerase deficiency
  - Pyruvate kinase deficiency
  - G-6-PD deficiency
- Disorders of red cell production
  - Diamond-Blackfan syndrome
  - Leukemia (usually associated with Down or Noonan syndrome)
  - Alpha-thalassemia (Bart hemoglobinopathy)
  - Parvovirus B19
  - Others
- Fetal hemorrhage
  - Placental subchorial tumors
  - Feto-maternal hemorrhage
  - Twin-to-twin transfusion
  - Isoimmune fetal thrombocytopenia
  - Others

Cardiovascular
- Structural anomalies
  - Abnormalities of left ventricular outflow
  - Abnormalities of right ventricular outflow
  - Other vascular malformations
- Non-structural anomalies
  - Obstruction of venous return
  - Supraventricular tachycardia
  - Congenital heart block
  - Prenatal closure of the foramen ovale or ductus arteriosus
  - Myocarditis
  - Idiopathic arterial calcification or hypercalcemia

Infectious
- Parvovirus B19
- Cytomegalovirus (CMV)
- Syphilis
- Herpes simplex
- Toxoplasmosis
- Hepatitis B
- Adenovirus
- Ureaplasma urealyticum
- Coxsackie virus type B
- Listeria monocytogenes

Genetic
- Inborn errors of metabolism
  - Glycogen-storage disease, type IV
  - Lysosomal storage diseases
  - Hypothyroidism and hyperthyroidism
  - Others
- Genetic syndromes
- Chromosomal syndromes
  - Beckwith-Wiedemann syndrome (trisomy 11p15)
  - Cri-du-chat syndrome (chromosomes 4 and 5)
  - Trisomy 10, mosaic
  - Trisomy 13
  - Trisomy 15
  - Trisomy 18
  - Trisomy 21 (Down syndrome)
  - Turner syndrome (45, X)
  - Others

Tumors and others
- Intrathoracic tumors or masses
- Abdominal tumors or masses
- Other conditions
  - Placental choriocarcinoma
  - Placental chorangioma
  - Cystic hygroma
  - Intussusception
  - Meconium peritonitis
  - Intracranial teratoma
  - Sacrococcygeal teratoma

Idiopathic

space (ascites, pleural effusion, or pericardial effusion), accompanied by skin edema (> 5 mm thick), or fluid in two potential spaces without edema [11, 12]. Ascites can be detected when a minimum of 50 mL is present in the fetal abdomen [13]. Polyhydramnios and placental thickening (> 6 cm thick) may be present, but oligohydramnios is a particularly ominous finding when it develops in non-immune hydrops fetalis [14].

The subsequent workup of the hydropic fetus should focus on detecting the underlying cause. In general, the first step is to collect detailed information about the mother's medical history, specifically in relation to hereditary or metabolic diseases, diabetes, anemia, exposure to infectious agents, and use of medication. The second step includes a detailed ultrasound examination of the fetus and maternal investigations. The third step, after obtaining maternal results, is a systematic approach to the fetus, including invasive testing, such as villocentesis, amniocentesis, cordocentesis and sampling of any effusions. Invasive investigations of the fetus are necessary when maternal bloods and ultrasound examination fail to provide a definitive cause of HF. The recommended workup of a fetus with HF is shown in Table 104.2.

If the etiology of HF is not identified before birth, postnatal investigations should be done. Blood samples for laboratory analysis are similar to those taken antenatally: blood group including Rh status, direct Coombs antibody screen, full blood cell count, karyotype, metabolic and chemistry studies, hemoglobin electrophoresis, if indicated. Structural defects should be evaluated using skeletal radiographs and ultrasound. A genetic consultation may also be helpful, particularly to determine risk of recurrence. In case of intrauterine or neonatal death, an autopsy is mandatory.

104.6 Treatment

The management of hydrops fetalis is a great challenge for fetal medicine specialists and neonatologists.

A woman with a hydropic fetus should be hospitalized in a level 3 perinatal centre if antenatal non-stress testing (NST)

Table 104.2 Antenatal evaluation of hydrops fetalis

Maternal history
- Age, parity, gestation
- Hereditary or metabolic diseases, anemia
- Recent infections or contacts
- Medication use

Maternal laboratory evaluation
- Complete blood cell count
- Blood type, Rh, indirect Coombs antibody screen
- Kleihauer-Betke stain
- Syphilis, TORCH and parvovirus B19 titers
- Culture for group B streptococcus, Listeria
- Maternal triple screen
- Oral glucose tolerance test
- Optional, as indicated:
  - Metabolic studies
  - Hemoglobin electrophoresis
  - G6PD, pyruvate kinase
  - Autoimmune screen (SLE, anti-Ro and -La)

Ultrasonography
- Identify anatomic abnormalities
- Evaluate extent of edema and effusions
- Exclude twin pregnancy
- Doppler blood flow studies

Fetal echocardiography
- Look for cardiac malformation, arrhythmia

Amniocentesis
- Karyotype
- Culture or PCR for TORCH, parvovirus
- Amniotic fluid alpha-fetoprotein
- Restriction endonucleases (thalassemias)
- Lecithin-sphingomyelin ratio, phosphatidyl glycerol to evaluate lung maturity

Fetal blood sampling
- Karyotype
- Complete blood cell count
- Blood type; hemoglobin electrophoresis
- Blood chemistry, albumin, gases
- Culture or PCR for TORCH, parvovirus
- Metabolic testing (Tay-Sacks, Gaucher, GM_1 gangliosidosis)

Fetal effusion sampling
- Culture or PCR for TORCH, parvovirus
- Protein content
- Cell count and cytology

G6PD glucose-6-phosphate dehydrogenase, *PCR* polymerase chain reaction, *SLE* systemic lupus erythematosus, *TORCH* toxoplasmosis, other agents, rubella, cytomegalovirus, herpes simplex. Modified from [10].

Table 104.3 Conservative management in hospital

- Hospitalize the patient in the presence of
  - Fetal skin thickening
  - Pericardial effusion
  - Nonreactive NST
  - Biophysical profile (BPP) ≤ 6
  - Subjective decreased fetal movement
  - Gestational age below 32–34 weeks
- Treat the underlying cause, if possible
- Administer antenatal corticosteroids
- Monitor serial growth and effusion volumes
- NST and BPP every 2–3 days

NST non-stress test.

[15] and biophysical profile (BPP) [16] are not reassuring (Table 104.3). At the same time, efforts should be continued to determine the underlying etiology of HF. Delivery is indicated after 34 weeks' gestation, or earlier if there is evidence of a mature fetal lung profile at amniocentesis or if the fetal condition deteriorates. Delivery is also necessary in cases of compromised maternal conditions due to the mirror syndrome (maternal hydrops) [17], a pre-eclampsia-like disease in mothers of hydropic fetuses.

The appropriate treatment of the fetus with hydrops, which carries a high mortality, can only be undertaken after a precise and detailed diagnosis. Full parental involvement is essential because the associated abnormalities may be severely debilitating or even lethal. In addition, invasive fetal treatment and elective preterm delivery remain controversial. Therefore obstetricians, fetal medicine specialists and neonatologists should consult about the optimal timing of delivery also involving pediatric surgeons, cardiologists, and cardiothoracic surgeons. Various anecdotal approaches are found in the literature, but no properly designed clinical trials have been done to provide the clinician with an evidence-base for management. Furthermore, the hydropic process may resolve spontaneously. Thus, management schemes aim to correct the underlying pathophysiology, including fetal transfusion to correct anemia (regardless of the cause), drug treatments for cardiac arrhythmias, correction or reduction of space-occupying lesions that impede cardiac venous or lymphatic return, and procedures intended to stop fetal blood loss (regardless of cause) [18–20].

Fetal transfusion with packed red blood cells (RBCs) given intraperitoneally has become accepted as standard care for the fetus with severe anemia. It carries low risk, even if there is no definitive evidence from randomized clinical trials. This approach has been used successfully in the treatment of severely anemic fetuses of isoimmunized pregnancies and to correct anemia due to various other causes. More recently, other routes (percutaneous umbilical vein, intrahepatic umbilical vein, umbilical artery, intracardiac transfusions) for the administration of blood products to the fetus have been reported. Other approaches have been aimed at the mother, fetus and newborn baby. Maternal plasmapheresis, promethazine or corticosteroids have been used for the mother. Fetal therapies have included partial packed-cell exchange transfusion, fetal intravenous IgG, platelet transfusion, and the administration of human granulocyte-stimulating factor. Neonatal stem cell transplantation has been used for α-thalassemia [21]. However, these newer therapeutic techniques have a greater risk to the fetus than the intraperitoneal route and should therefore be used cautiously.

Highly vascular tumor masses and acute, massive twin-to-twin hemorrhages are life-threatening diseases that may justify life-threatening treatment. Techniques such as tumor debulking surgery, surgery for active bleeding, photocoagulation

and radiofrequency thermal ablation may all be helpful in the treatment of fetal conditions such as sacrococcygeal tumors, highly vascularised fetal intraabdominal, thoracic, or placental masses, and when there is massive arteriovenous shunting [22–23].

The management of the twin-to-twin transfusion syndrome is currently an unresolved problem: treating an anemic fetus with transfusions has shown no evidence of benefit; volume reduction for the transfusion recipient or a combination of transfusion and fetal reduction has rarely been used or may not correct the ongoing pathophysiology. Furthermore, feticide of the affected twin is often followed by the development of hydrops in the previously normal surviving twin [24].

Treatments of fetal arrhythmias include doing nothing, drugs, and immediate delivery. In the presence of fetal maturity, the simplest and most direct approach is delivery of the affected fetus and treatment of the arrhythmia directly after birth. If this is not possible, drugs have been used. Drugs have been administered to mother, or fetus, or both. Medications have included digitalis, furosemide, flecainide, verapamil, amiodarone, propranolol, procainamide, quinidine, adenosine, sotalol, terbutaline, corticosteroids, and immunoglobulins. Various drug combinations have also been used. However, the choice of the drug remains empirical and arbitrary, until definitive evidence from clinical trials becomes available [25, 26].

The management of space-occupying masses varies depending on the type of lesion and from centre to centre. If immediate delivery is not practicable, the mass is either reduced or removed. Pleural and pericardial effusions and ascites have been treated with single or serial drainage. Fetal surgery with definitive correction of the underlying anomaly has also been used. Successes and failures have been reported with all methods; there is no evidence suggesting that one approach is better than another [27].

Postnatal management of HF poses a unique set of problems for the neonatologist. Treatment of the infant after delivery is helped by knowing the cause. In addition to appropriate equipment and supplies for resuscitation, a skilled team of health care professionals (neonatologists, nurses, respiratory therapists, radiograph and ultrasonography technicians) should be present in the delivery room [28, 29]. Fluid in the pleural, pericardial and abdominal cavities may require aspiration in the delivery room to allow adequate ventilation and circulation. Umbilical arterial and venous catheters are sited to monitor and treat arterial pressure, blood gases, venous pressure, hematocrit and the metabolic state of the infant. Packed red cells or whole blood cross matched with the mother should be available for the correction of severe anemia by partial exchange transfusion, even when due to non-immune causes. Surfactant therapy and mechanical ventilation are used to manage surfactant deficiency and pulmonary hypoplasia, which may be associated with hydrops. Fluid intake is based on an estimate of the infant's "dry weight" (e.g., 50th percentile for gestational age) and kept to a minimum (e.g., 40–60 mL/kg/day) until the edema is resolved. Inotropic support (e.g., dopamine) may be required to improve cardiac output [30, 31].

104.7 Prognosis

Estimates of mortality vary widely. The condition has a mortality rate of virtually 100% when structural defects are present or the cause of HF is unknown. Most case series report 60–90% mortality, although notable improvements have been described in more recent reports [5]. In cases of tachyarrhythmias, the prognosis has been improved by antenatal antiarrhythmic treatment. Cases presenting before 24 weeks have a worse prognosis [32], whereas those that present later may benefit from delivery and intensive neonatal care. The risk of recurrent hydrops in a subsequent pregnancy is low, although one series reported a 10% risk of recurrence [18]. In conclusion, despite the fetus with hydrops having profoundly compromised perfusion and impaired function of multiple organ systems, the limited follow-up data that is currently available provides an optimistic outlook for babies who survive fetal hydrops [33].

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105

Physiology and Abnormalities of Leukocytes

Kurt R. Schibler

105.1 Physiology of Leukocytes in the Fetus and Neonate

105.1.1 Introduction

The circulating pool of neutrophils reflect a dynamic equilibrium between neutrophil precursors and mature neutrophils in the bone marrow, circulating neutrophils and activated neutrophils that have migrated into the tissues. The bone marrow is the predominant hematopoietic organ after birth. Within the bone marrow, pluripotential hematopoietic stem and progenitor cells give rise to lineage committed neutrophil precursors. These precursors mature under the influence of growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF). The bone marrow thus contains pools of immature neutrophils and of stored mature neutrophils. After leaving the bone marrow, neutrophils are transiently in the circulation for a brief period of hours after which they translocate into the tissues.

The peripheral blood neutrophil pool is estimated to contain approximately 5% of the total neutrophil pool. Approximately 1.5 billion neutrophils per kilogram body weight are produced daily. The neutrophil life span consists of about 9 days in the bone marrow, 3–6 hours in the blood, and 1–4 days in the tissues.

Myeloblasts, promyelocytes, and myelocytes are collectively referred to as the neutrophil proliferative pool (NPP) because these cells retain the capacity for cell division. As committed neutrophil precursors mature, their capacity to undergo cell division is lost. This population of postmitotic cells includes metamyelocytes, band neutrophils and mature segmented neutrophils, and is called the neutrophil storage pool (NSP). Neutrophils are released from the NSP to maintain the circulating and marginated neutrophil pools in equilibrium. About 0.3×10^9 neutrophils per kilogram body weight are present in the circulating pool of healthy adults [1].

Adults have a large NSP and can increase circulating neutrophils rapidly in response to infection [2]. By contrast, newborn infants have a relatively small NSP [2]. These differences in the available pool of postmitotic neutrophils limit the ability of newborn infants to respond to the challenges of infections. The circulating neutrophil concentration in humans is relatively low during gestation, but progressively rises until term. Postnatally, the circulating neutrophil count is much higher. Manroe and colleagues described neutrophil ranges in normal term infants during the first 28 days of life [3]. They demonstrated a rise in neutrophil count with a peak between 12 and 24 hours and neutrophil concentrations at that time between 7800 and 14,500 cells per microliter. The neutrophil count subsequently decreased and stabilized after 72 hours with a lower value of 1750 cells per microliter. Mouzihno and coworkers established a reference range for blood neutrophil concentrations in very low birth weight infants [4]. The upper range for normal neutrophil concentration was similar to that of term infants. The lower levels, defining neutropenia, were about 2000 cells per microliter at 12 hours and stabilized at 1000 cells per microliter by 48 hours of life.

The normal circulating monocyte count in the healthy human adult is estimated to be between 300 and 450 monocytes/mm^3. Peripheral blood monocyte counts have been observed to vary in a cyclic fashion over a 3–6 day period [5, 6].

The promonocyte cell cycle rate varies between 30 and 48 hours. These rapidly dividing cells achieve a production rate between 7×10^5 and 7×10^6 monocytes/kg of body weight per hour. After 60 hours of maturation in the bone marrow, mature monocytes move into the intravascular space, where they circulate for approximately 3 days [7]. Van Furth and coworkers [8] calculated that 1.66×10^{10} circulating monocytes leave the intravascular space/hour.

Tissue macrophages are derived from bone marrow precursors. The first evidence for the bone marrow origin of

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macrophages was obtained in mouse chimeras [9] and rat parabiosis experiments [10, 11]. Studies in patients undergoing bone marrow transplantation, isolation and characterization of lung [12] and liver macrophages [13], provided evidence that these cells contained the karyotype of the bone marrow donor. Thus, terminally differentiated macrophages must have been derived from transplanted bone marrow. Furthermore, by following the disappearance of the Y body–containing macrophages from male patients who have received female donor marrow, the half-life of the alveolar macrophage was estimated to be 81 days.

The size of the tissue macrophage compartment is considerable. According to some estimates, the number of tissue macrophages is 500–1000 times greater than the bone marrow compartment. Some organs, such as the lung and liver, may have particularly large macrophage populations that may account for between 20 and 30% of the total cell number. These cells are long lived and have been estimated to survive for several months [12–14].

105.1.2 Leukocyte Functional Differences Associated with Prematurity

After neutrophils have emigrated from the circulation, cell movement may occur either by random movement or by directed movement toward a chemoattractant. Random movement not related to a stimulus occurs in any direction with equal probability. The ability to undergo directed migration depends upon sensory mechanisms capable of detecting differences in concentration of chemotactic molecules and linking this information to the locomotory apparatus in the cell.

Random movement by neonatal neutrophils is normal compared to that observed in adult cells [15]. However, a number of studies indicate that directed movement of neutrophils from preterm and term neonates to defined chemotactic stimuli is impaired [16–18].

Mononuclear phagocytes exhibit both random and directed movement. Random or nondirected movement occurs in the absence of attracting substances. Directed movement along a concentration gradient of chemical attractants is essential for the effective localization of mononuclear phagocytes to sites of infection and inflammation. This directional movement, called chemotaxis, is governed by chemoattractant molecules that bind to receptors on the cell surface. Chemotactic substances are produced through activation of the complement, fibrinolytic and kinin systems or are directly produced by microorganisms. These chemoattractants may also accentuate random migration of mononuclear phagocytes in the absence of a concentration gradient. An entire family of proinflammatory molecules called chemokines has been identified and many of its members have been characterized [18]. Several members of this family serve as potent chemoattractants for mononuclear phagocytes. Monocyte chemotactic protein 1, macrophage inflammatory protein 1, and RANTES (regulation upon activation, normal T-cell expressed and secreted) are key regulators of monocyte migration. Monocyte-derived dendritic cells migrate to lymphoid organs in response to secondary lymphoid chemokine [19, 20].

Mononuclear phagocyte migration has been examined in cord blood and neonatal peripheral blood. In most reports, random movement by cord blood monocytes appears to be equivalent to that of adult peripheral blood monocytes [21–23]. By contrast, several investigators have reported diminished directed movement by monocytes from cord and neonatal peripheral blood compared with those from adult peripheral blood. Yegin [24] reported that monocyte chemotaxis increased gradually during childhood and was equivalent to adult chemotactic activity at 5–6 years of age. These results are consistent with those of other investigators who reported similar chemotactic activity by cord blood and adult peripheral blood monocytes, but dramatically decreased chemotaxis by neonatal peripheral blood monocytes in the first few days of life [25]. Monocyte chemotaxis gradually increased but remained lower than adult peripheral blood monocyte activity during the first 6 months of life. Other groups of investigators reported slightly increased chemotaxis of cord blood monocytes in response to endotoxin-activated adult serum or activated adult lymphocytes [15, 26]. Differences in methods of measurements applied, patient populations examined and monocyte isolation used, likely account for the contradictory results reported by these investigators.

Neutrophils establish contact with microorgnisms through opsonins, including complement and IgG antibody. The phagocyte completely encircles the microorganisms with circumferential interactions between opsonins and receptors. Once the newly formed vacuole is internalized, the products of intracellular granules are discharged into the vacuole [27, 28]. Mobilization of granules appears to be normal in newborns compared to adults [29]. Neutrophilic granules from newborn infants possess equivalent content of myeloperoxidase and lysozyme, but diminished quantities of lactoferrin and gelatinase [29, 30].

In several experiments, neutrophils from healthy term infants provided with opsonins from adult serum exhibited normal phagocytosis of a variety of microorganisms including *Escherichia coli*, group B Streptococci, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, and *Candida albicans* [31–34]. Phagocytosis by neutrophils from preterm infants, while less well studied, appears to have competent phacocytic functions for bacteria and foreign particles. Ingestion of *Candida albicans* by neutrophils of preterm infants was reported to be normal in one study, but diminished in others [34–36].

The kinetics of phagocytosis by monocytes from cord blood and adult peripheral blood have been studied by Schuit and Powell [37]. These investigators found that monocytes isolated from adult peripheral blood ingested all polystyrene particles within 50 minutes. However, only 38% of cord

blood monocytes had initiated phagocytosis in the same time period. The cord blood monocytes were able to ingest the particles by 100 minutes. These studies were not influenced by the presence of serum or heat-inactivated serum. Other investigators have reported that opsonized (IgG) sheep erythrocytes, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Toxoplasma gondii* and type II herpes simplex are ingested by cord blood monocytes as effectively as monocytes derived from adult peripheral blood [38]. Defective phagocytosis of *Streptococcus agalactiae* by cord blood monocytes has been reported by Marodi and colleagues [39]. Monocytes derived from term cord blood ingested fewer group B streptococci than monocytes isolated from adult blood. Other studies suggested that the phagocytic defect of group B *Streptococcus* by cord blood monocytes could be overcome by inclusion of adhesive glycoprotein fibronectin in the culture. Although fibronectin alone did not enhance phagocytosis, there was enhanced phagocytosis by cord blood monocytes of group B *Streptococcus* preopsonized with IgG preparation in combination with fibronectin.

The phagocytic activity of cord blood monocyte–derived macrophages has been compared with that of macrophages derived from adult blood monocytes. After a 10 day culture period, Speer and co-workers [40] determined that cord blood–derived macrophages ingested complement opsonized *S. aureus* to the same extent as macrophages from adult blood. Other investigations compared the phagocytic activity of alveolar macrophages from intubated newborns with alveolar macrophages from bronchoalveolar lavage of adult subjects. Phagocytosis of *C. albicans* by newborn and adult alveolar macrophages was determined to be equivalent with respect to rate and number of organisms ingested [41].

Table 105.1 Frequency of leukocyte abnormalities

Common
- Bacterial and fungal infection
- Maternal hypertension

Moderately common
- Twin-twin transfusion
- Isoimmune
- Rh hemolytic disease
- Drug induced
- Viral infection (rubella, cytomegalovirus)
- Leukaemoid reaction

Rare
- Severe congenital neutropenia (Kostmann syndrome)
- Cyclic neutropenia
- Shwachman-Diamond syndrome
- Reticular dysgenesis
- Autoimmune neutropenia
- Chediak-Higashi syndrome
- Dyskeratosis congenita
- Cartilage-hair hypoplasia syndrome
- Chronic granulomatous disease
- Leukocyte adhesion deficiency types I and II

105.2 Leukocyte Abnormalities

105.2.1 Etiology and Pathogenesis

The chief disorders of leukocytes observed in the newborn period include quantitative abnormalities such as neutropenia, neutrophilia, and a leukemoid reaction. Qualitative defects in phagocytic leukocytes also occur in conjunction with quantitative abnormalities and with rare hematopoietic disorders. Leukocyte disorders that occur in the neonatal period are categorized based on the frequency of the condition in Table 105.1.

105.2.2 Common Leukocyte Abnormalities

105.2.2.1 Infection

Animal studies comparing the hematopoietic response to infection between neonates and adults have demonstrated differences in the supply and release of neutrophils from the bone marrow [42]. These developmental differences increased the susceptibility of newborn animals to depletion of bone marrow reserves when subjected to experimental infection and ultimately limited their capability to survive such infections [43, 44]. Septic neonates who are neutropenic have a higher mortality than non-neutropenic neonates [45, 46]. Neonates also have an immaturity of neutrophil function and production. Immaturity of granulopoiesis in preterm neonates is manifest by a low neutrophil cell mass, a reduced capacity for increasing progenitor cell proliferation and frequent occurrence of neutropenia in response to sepsis [47]. Neonatal neutrophil function may be reduced in signal transduction, cell surface receptor upregulation, mobility, cytoskeletal rigidity, microfilament contraction, oxygen metabolism and intracellular oxidant mechanisms [48].

105.2.2.2 Associated with Maternal Hypertension

A high proportion of newborn infants delivered to women with hypertension have reduced circulating neutrophil concentrations [3, 4] because of decreased neutrophil production [49], particularly when there is fetal growth restriction. Studies have shown a decrease in neutrophil progenitor cells and decreased cycling of these cells, a relatively normal NSP, and absence of immature neutrophils in the circulation, termed a left shift. Although the precise cause of reduced neutrophil production is uncertain, it does not appear to be related to maternal medications [49, 50]. Non-specific innate host defenses may be impaired and there is a higher incidence of nosocomial infection among infants delivered to hypertensive mothers neutropenic infants compared to non-neutropenic infants [49, 51, 52].

105.2.3 Moderately Common Leukocyte Abnormalities

105.2.3.1 Leukemoid Reaction

Newborn infants may mount an exaggerated response to infection with an incease in neutrophil count and a left shift. There is a significant increase in neutrophil precursors in the peripheral blood as well as an increase in the WBC count exceeding 50,000 cells per microliter is considered a leukemoid reaction. A leukemoid reaction in response to a severe infection in the newborn is frequently accompanied by cytoplasmic vacuoles within the neutrophils and by the appearance of toxic granulations. Infants with Down syndrome may have a type of leukemoid reaction unrelated to infection.

105.2.3.2 Associated with Rh Hemolytic Disease

A well-documented complication of severe Rh erythroblastosis fetalis is a reduction in the concentration of neutrophils and platelets (35, 36). In one series of 20 infants with Rh sensitization, 11 infants had severe disease requiring multiple exchange transfusions (59). Before exchange transfusion all infants with severe disease had neutropenia. The neutrophil kinetic defect persisted between 3 and 5 days in these infants whereas none of the infants with mild disease exhibited neutophil depletion.

105.2.3.3 Isoimmune Neonatal Neutropenia

Isoimmune neonatal neutropenia results from maternal production of IgG directed against antigens on fetal neutrophils (2). This is analogous to Rh hemolytic disease in that maternal sensitization to fetal neutrophil antigens results in transplacentally acquired IgG antibody that destroys the infant's neutrophils (37). Maternal sensitization may occur during gestation and may even affect the fetus of a primagravida (38). The incidence of isoimmune neonatal neutropenia is estimated to be 0.5–2 per 1000 live births (39).

Affected infants frequently develop a fever in the first days of life and are particularly susceptible to cutaneous infections caused by *Stapylococcus aureus*. β-hemolytic *Streptococcus* and *E. coli* have also been linked to infections among susceptible infants with this disorder. The onset of infection is usually concurrent with severe neutropenia. In the circulation, the concentrations of other myeloid lineages particularly monocytes and eosinophils are typically increased. Characteristic findings on bone marrow examination are myeloid hyperplasia with a paucity of mature neutrophils and normal erythropoietic and megakaryocytic elements.

Neutrophil antibodies are detected in the sera of mother and infant. The antibodies react against neutrophils of the patient and of the father, but not against neutrophils from the mother. Several neutrophil-specific antibodies have been implicated including most commonly human neutrophil alloantigens (HNA)-1, HNA-2, and HNA-3. Other antingenic targets include NC1, SH, SAR, LAN, LEA, CN1, and certain HLA antigens. HNA-1 and NC1 have been identified as isotypes of the FcγIII receptor (2). HNA-2 is an antigen on glycoprotein (GP)50 and HNA-3 corresponds to an antigen on GP75-90. The infant's neutrophil counts typically normalize over the first 1–5 weeks of life as might be expected with the half-life of maternal antibodies.

Treatment for affected infants is supportive. Therapy also includes appropriate antibiotic administration for infections and close follow-up. The use of prophylactic antibiotics has been shown to be ineffective. Intravenous immunoglobulin (IVIG) administration and steroid therapy have not been shown to consistently improve circulating neutrophil counts. For infants with persistence of extremely low neutrophil counts (less than 500 cells per microliter), G-CSF administration might be undertaken. This therapy usually results in a prompt clinical response in circulating neutrophil concentrations.

105.2.3.4 Neonatal Autoimmune Neutropenia

Neonatal autoimmune neutropenia results from transplacental passage of maternal IgG autoantibodies directed against neutrophil antigens. Mothers of these infants can be asymptomatic or have autoimmune neutropenia from systemic lupus erythematosis or idiopathic thrombocytopenic purpura (40).

105.2.3.5 Drug-Induced Neutropenia

A significant number of medications have been implicated as causes of the development of neutropenia (Table 105.2). The mechanisms may involve bone marrow suppression or immune-mediated destruction. Marrow recovery generally begins several days after discontinuing the causative agent.

105.2.3.6 Late-Onset Neonatal Neutropenia

Most episodes of neonatal neutropenia occur during the first week of life and are related to low gestational age, low birth weight, infections, maternal hypertension, severe neonatal asphyxia, drug therapy or other perinatal events, or are of unknown cause. By contrast, late-onset neonatal neutropenia, defined as an absolute neutrophil count (ANC) $<1500/mm^3$, is observed in normally growing very-low birth-weight (VLBW) infants at a postnatal age of ≥3 weeks and is not associated to the typical complications that usually precede early-onset neonatal neutropenia (41).

Although less frequent than the early-onset type, late-onset neutropenia may be found in a significant proportion,

Table 105.2 Examples of drugs associated with neutropenia

Anti-inflammatory agents
- Indomethacin
- Pentazocine
- Para-aminophenol derivatives
  - Acetoaminophen
- Pyrozolone derivatives
  - Aminopyrine
  - Dipyrone
  - Oxyphenbutazone
  - Phenylbutazone

Antibiotics
- Cephalosporins
- Clindamycin
- Gentamicin
- Isoniazid
- Penicillins and semisynthetic penicillins
- Rifampin
- Streptomycin
- Trimethoprim-sulfamethoxazole
- Vancomycin

Anticonvulsants
- Carbamazepine
- Phenytoin
- Sodium valproate

Antidepressants
- Amitriptyline
- Amoxapine
- Doxepin
- Imipramine

Antihistamines (H-2 Inhibitors)
- Cimetidine
- Ranitidine

Antimalarials
- Amodiaquine
- Chloroquine
- Dapsone
- Pyrimethamine
- Quinine

Antithyroid
- Carbimazole
- Methimazole
- Propylthiouracil

Cardiovascular
- Captopril
- Dispopyramide
- Hydralizine
- Methyldopa
- Procainamide
- Propranolol
- Quinidine

Diuretics
- Acetazolamide
- Chlorthiadone
- Chlorothiazide
- Ethicrynic acid
- Hydroclorothiazide

Hypoglycemic agents
- Chlorpropamide
- Tolbutamide

Sedatives
- Benzodiezapines
- Meprobamate

Phenothiazines
- Chlorpromazine
- Phenothiazines

up to 22% of VLBW and in 5.5% of low birth-weight (LBW) infants, and is associated with anemia of prematurity and high reticulocyte counts, or the administration of erythropoietin at very high doses (42).

Mean age at onset of neutropenia is about 6 weeks, while the duration may range from 2–7 weeks.

An imbalance between hematopoietic growth factors (mainly erythropoietin and granulocyte colony-stimulating factor) with increased reticulocytopoiesis in response to anemia may explain the neutropenia.

Late onset neutropenia appears to be a benign condition that is not associated with any particular complication and does not require specific treatment. However, investigation should be performed to exclude other causes of neutropenia.

105.2.4 Rare Leukocyte Abnormalities

Hereditary neonatal neutropenia is covered elsewhere (see Chapter 106).

15.2.4.1 Cartilage-Hair Hypoplasia Syndrome

Cartilage-hair hypoplasia is an autosomal recessive disorder characterized by short-limbed dwarfism, fine hair, and moderate neutropenia [53]. Affected individuals have an increased susceptibility to infections, particularly varicella zoster viral infections. Immunologic defects are variable, but cellular immune functions are impaired.

105.2.4.2 Reticular Dysgenesis

Reticular dysgenesis, also called congenital aleukocytosis, is characterized by severe neutropenia associated with leukopenia, presence of rudimentary thymic lymphoid and splenic tissue, and agammaglobulinemia [54]. Histological examination of the bone marrow, spleen and lymphoid tissue reveals normal reticular structure with normal erythroid and megakaryocyte elements, but with absent or sparse myeloid cells. The mechanism of the disorder is unknown, but a defect in lymphohematopoietic progenitor cell development is suspected [55]. Bacterial and viral infections occur early in life and are severe. Aggressive antibiotic therapy and supportive care are necessary for survival. Treatment with G-CSF and GM-CSF have been ineffective. Bone marrow transplantation remains the only long-term treatment option available for infants with this disorder [54, 56].

105.2.4.3 Dyskeratosis Congenita

Dyskeratosis congenita (DC) is an inherited bone marrow failure syndrome clinically characterized by the triad of abnormal nails, reticular skin pigmentation, and oral leukoplakia, and is associated with high risk of developing aplastic anemia, myelodysplastic syndrome, leukemia, and solid tumors. Patients have very short germline telomeres, and approximately half have mutations in one of six genes encoding proteins that maintain telomere function. Accurate diagnosis of DC is critical to ensure proper clinical management, because patients who have DC and bone marrow failure do not respond to immunosuppressive therapy and may have increased morbidity and mortality associated with hematopoietic stem cell transplantation [57].

105.2.4.4 Leukocyte Adhesion Deficiency Type I

Leukocyte adhesion deficiency (LAD) encompasses a rare group of autosomal recessive disorders [58]. LAD type I is associated with defects in neutrophil adhesion and chemotaxis resulting in impaired clearance of complement-opsonized microorganisms. Infants present with delayed separation of the umbilical cord and recurrent infections despite a normal or increased neutrophil count. LAD should be suspected in any

infant with unusually severe bacterial infections accompanied by normal or increased circulating neutrophils and a striking absence of purulent material at the site of infections.

The clinical presentation varies depending on the relative deficiency of CD18. CD18 is the β_2-subunit of the cell surface leukocyte integrin. These molecules are involved in adherence, chemotaxis, C3bi-mediated ingestion, degranulation, and neutrophil respiratory burst. Diagnosis of LAD type I is made by demonstration of a severe deficiency of the β_2-subunit of neutrophil integrins. Carriers can be identified as having about 50% of the normal β_2-subunit levels in circulating neutrophils, whereas affected individuals express 0–10% levels. α/β-Integrins are common on the surface of all neutrophils and make up three distinct cell surface proteins: LFA-1 ($\alpha_1\beta_2$), MAC-1 ($\alpha_m\beta_2$) and P150, 95 ($\alpha_x\beta_2$). Each of these integrins are affected because they require a common β_2-subunit that is diminished in quantity or functionally defective. Several mutations have been identified in the gene coding for the β_2-subunit. These mutations fall into several categories in which there are absent, low or normal level of CD18 mRNA and in which the β_2-subunit protein precursor is absent, low in abundance or abnormal in size. There is a close genotype-phenotype correlation between the molecular defect and the clinical severity of disease.

Leukocyte trafficking from bloodstream to tissue is important for the continuous surveillance for foreign antigens as well as for rapid leukocyte accumulation at sites of inflammatory response or tissue injury. Leukocyte interaction with vascular endothelial cells is a pivotal event in the inflammatory response and is mediated by several families of adhesion molecules. The crucial role of the beta(2)-integrin subfamily in leukocyte emigration was established after LAD type I was discovered. Patients with this disorder suffer from life-threatening bacterial infections, and, in its severe form, death usually occurs in early childhood unless bone marrow transplantation is performed.

The LAD type II disorder (described in more detail below) clarifies the role of the selectin receptors and their fucosylated ligands and patients have a less severe form of infectious episodes (resembling the moderate phenotype of LAD type I) but also severe psychomotor and growth retardation. LAD type III emphasizes the importance of the integrin-activation phase in the adhesion cascade and all hematopoietic integrin activation processes are defective, leading to severe infection as observed in LAD type I and to an increased tendency for bleeding problems due to defective activation of beta(1), beta(2), and beta(3) integrins [58].

105.2.4.5 Leukocyte Adhesion Deficiency Type II

LAD type II is a rare autosomal recessive clinical syndrome involving recurrent bacterial infections, short stature, severe mental retardation, and the hh (Bombay) RBC phenotype. Neutrophils from patients affected with this disorder exhibit markedly diminished chemotaxis *in vitro*, although the neutrophils display normal levels of CD18 and are able to ingest serum-opsonized particles. The molecular defect in these patients is a defect in the fucosyltransferase gene responsible for the carbohydrate linkages associated with the AB blood groups, specifically the sialyl-Lewis X structure. Neutrophil function is defective in these individuals because sialyl-Lewis X is the neutrophil cell surface ligand recognized by endothelial cell surface E-selectin and P-selectin receptors [59].

105.2.4.6 Chediak-Higashi Syndrome

Chediak-Higashi syndrome is a rare, autosomal recessive disorder characterized by the presence of giant cytoplasmic granules in multiple cells throughout the body. The syndrome affects neutrophil and lymphocyte function [60]. Defects in the CHS1 gene family have been implicated in the causation of this disorder. These genes encode proteins involved in vesicular trafficking although the precise function of these proteins is uncertain. Neutrophils display abnormal adherence and chemotaxis, delayed degranulation, and impaired ingestion and killing of microorganisms. Patients with this syndrome are highly susceptible to bacterial infections. Most patients with Chediak-Higashi syndrome exhibit lymphohistiocytic infiltration of multiple organs that appears to result from a lack of natural killer cell function. Death often occurs in the first decade of life from infection, bleeding, or lymphohistiocytic infiltration. Bone marrow transplantation can be effective in treating the hematopoietic complications of Chediak-Higashi syndrome.

105.2.4.7 Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is an inherited immunodeficiency syndrome in which generation of superoxide by the respiratory burst oxidase of phagocytic leukocytes is markedly diminished or absent [61]. The disorder, which has an incidence of approximately one per 500,000 individuals, results from mutations in any one of four essential subunits of the respiratory burst complex. Superoxide generated during the respiratory burst is the precursor for a number of potent oxidants that are important in elimination of many microbial pathogens. Approximately two thirds of CGD cases are caused by defects in the X-linked gene encoding gp91-phox, the large subunit of the flavocytochrome b_{588}, a plasma membrane heterodimer that is the redox center of the phagocyte oxidase [61, 62]. A rare autosomal recessive form of CGD is caused by mutations in the gene encoding the small subunit of the p22-phox flavocytochrome b_{588}. Other cases of autosomal recessive CGD are caused by gene defects in two soluble proteins that interact with flavocytochrome b_{588}, p47-phox or p67-phox.

The clinical manifestations of CGD are apparent during infancy or early childhood [61–64]. Patients with CGD

experience recurrent purulent bacterial and fungal infections that are difficult to treat and often may be life threatening. Common sites of infection are skin, lymph nodes, lungs, bones, liver, and the gastrointestinal tract. Individuals with CGD are particularly susceptible to infections with *S. aureus*, a variety of gram-negative bacilli including *Pseudomonas*, *Salmonella*, and *Serratia* species and fungus such as *Aspergillus* species. Many of these microorganisms express catalase, which prevents phagocytes of CGD patients from scavenging microbe-generated hydrogen peroxide to promote microbial killing within the phagosome. The hallmark of CGD is the propensity to develop chronic inflammatory granulomas with wide spread tissue distribution [63, 65].

The prognosis for individuals with CGD has improved significantly since the disorder was first described in the 1950's. Current management includes aggressive treatment of acute infections in combination with the use of prophylactic antibiotics and interferon-γ (IFN-γ) [66, 67]. Allogenic bone marrow transplantation can be curative even in cases where transplantation results in mixed chimerism [68–70].

Because the genetic defect of CGD primarily affects cells of the hematopoietic system, this disorder has been considered a prime candidate for gene therapy targeted at hematopoietic stem cells [71]. Insertion of a functional copy of the involved gene into hematopoietic stem cells should reconstitute the respiratory burst oxidase activity in circulating and tissue phagocytes and thus could provide a life-long cure of the disease.

105.2.4.8 Autoimmune Neonatal Neutropenia

Autoimmune neutropenia (AIN) is a disorder caused by increased destruction of neutrophils as a result of autoantibodies to the patient's own neutrophils. The incidence is approximately one per 100,000 live births [72]. Primary AIN is not associated with other autoimmune disorders such as systemic lupus erythematosus. While this disorder was definitively identified in 1975, its cause has not been identified [73, 74]. Reports of associations with parvovirus B19 and beta-lactam antibiotics suggest mechanisms such as development of cross-reacting antibodies resulting from molecular mimicry, changes in endogenous antigens, enhanced HLA expression, or loss of suppression of self-reacting lymphocyte clones. HNA-1a (NA-1) autoimmunization has been linked to HLA-DR2, a finding implicating immune response genes [2]. Patients with this immune mediated neutropenia present in the first 3 years of life with a variety of infections. In approximately 80% of patients the infections are mild, consisting of abscesses, conjunctivitis, gastroenteritis, otitis media, pyoderma, and upper respiratory infections. In the remaining cases, AIN predisposes to serious infections such as meningitis, pneumonia, and sepsis [75]. Diagnosis of AIN is made by detection of neutrophil-specific antibodies. Although patients with AIN do not generally require specific treatment for neutropenia, to increase neutrophil counts in cases of severe infection or before elective surgery, some patients have been treated with corticosteroids, IVIG or G-CSF. Corticosteroids and IVIG increased neutrophil counts in about 50% of individuals, whereas G-CSF increased neutrophil counts in all patients treated. Infections are treated symptomatically with antibiotics. Infants with recurrent infections are often treated with prophylactic antibiotics.

105.2.5 Clinical Aspects

Classification of leukocyte abnormalities according to the underlying kinetic mechanism can provide useful clues for diagnosis and management of affected infants. The complete blood count and differential white count provides an estimate of the responsible kinetic mechanism by determining the degree of left shift, or the ratio of immature to total neutrophils in the blood. Neutropenia accompanied by a large left shift is likely to be on the kinetic basis of accelerated neutrophil destruction or usage [44, 76]. A large left shift is defined as a ratio of immature (band neutrophils or metamyelocytes) to total neutrophils of greater than 0.3. By contrast, neutropenias in which only mature neutrophils are observed in the blood are more likely to be on the kinetic basis of diminished neutrophil production or excessive margination [49, 77–79].

The variety of leukocyte abnormality is often suggested by associated findings (Table 105.3). For instance, neutropenia in an infant with intrauterine growth restriction delivered to a mother with hypertension, is likely to have neutropenia based on diminished neutrophil production, as commonly seen in that condition [49, 78]. The presence of neutropenia in the mother of an infant with neutropenia should prompt evaluation for maternal autoimmune antibodies causing immune-mediated neutrophil destruction. The presence of steatorrhea in a neutropenic neonate should prompt consideration of Shwachman-Diamond syndrome [80–82]. Severe and prolonged neutropenia, with counts typically less than 200 cells per microliter associated with monocytosis and eosinophilia

Table 105.3 Associated findings in newborn patients with leukocyte abnormalities that can suggest a specific disorder

Associated Finding	Variety of Leukocyte Abnormality
Albinism	Chediak-Higashi syndrome
Anemia and multiple gestation	Donor in twin-twin transfusion
Blueberry muffin rash	Congenital cytomegalovirus
Down syndrome	Leukemoid reaction
Hydrops	Rh hemolytic disease
Monocytosis and eosinophilia	Kostmann syndrome
Mother with hypertension	Neutropenia of PIH
Mother with neutropenia	Maternal autoimmune neutropenia
Pancytopenia	Reticular dysgenesis
Shock	Endotoxemia or sepsis
Steatorrhea	Shwachman-Diamond syndrome

on peripheral blood smear is seen in Kostmann syndrome where bone marrow examination is characterized by arrested neutrophil development at the promyelocyte or myelocyte stage [83, 84]. Neutrophilia with immature circulating myeloid elements in a stable infant with Down syndrome should prompt long-term follow-up because, although the neutrophilia is generally transient, up to 30% of these infants may develop leukemia later in childhood [85]. Neutrophilia or neutropenia with a left shift in conjunction with clinical signs of shock will prompt early and aggressive antibiotic treatment and supportive care in addition to investigation to identify the causative microbial agent. An association of neutrophilia and the presence of a "blueberry muffin rash", which is often indicative of extramedullary hematopoiesis, might prompt evaluation for congenital viral infections such as cytomegalovirus (CMV).

105.2.6 Differential Diagnosis

When evaluating a neonate with leukocyte abnormalities it can be helpful to maintain the perspective that some varieties of these abnormalities are common and others are exceedingly rare. The most common causes of leukocyte abnormalities among infants have an obvious underlying cause. One form of neutropenia, where there is a large left shift or an immature to total neutrophil ratio exceeding 0.3–0.5, accompanies overwhelming sepsis. However, another form of neutropenia (generally with no left shift or an immature to total neutrophil ratio of less than 0.2) occurs in infants with intrauterine growth restriction born to a woman with pregnancy-induced hypertension. In general these common varieties of neutropenia do not require additional investigations. Neutropenia accompanying sepsis resolves quickly if the patient survives. However, if neutropenia in the infant with sepsis persists for several days, additional investigations should be considered. Similarly, in most cases of neutropenia accompanying pregnancy-induced hypertension, neutropenia resolves within 5 days. If neutropenia persists for more than 5 days additional investigations may be considered, particularly if the neutrophil count is less than 500 cells per microliter.

Laboratory tests to be considered are those that provide a specific diagnosis, such as alloimmune or autoimmune neutropenia. These tests, while not diagnostic, are required to investigate rare forms of neutropenia such as congenital and cyclic neutropenia. Obtaining a complete blood count on the infant, including microscopic examination of neutrophil morphology, can be useful in identifying features such as left shift, monoytosis, eosinophilia, and the presence of cytoplasmic inclusions formed by fused lysosomes. Monocytosis and eosinophilia are commonly observed in patients with congenital neutropenia and cytoplasmic inclusions and are characteristic of Chediak-Higashi syndrome [60]. Also, a complete blood count on the mother to establish her neutrophil concentration can be useful to detect cases of autoimmune neutropenia in which mother has immune-mediated neutropenia, which is passively acquired by the fetus. Maternal and infant neutrophil antigen typing and anti-neutrophil antibody determination should identify most cases of immune-mediated neutropenia.

To evaluate neonatal immune mediated neutropenia, antibody testing is performed on both maternal and infant blood samples including two screening tests: 1) a neutrophil agglutination assay and 2) a granulocyte immunofluorescence assay. If either assay is positive, an HLA screen is performed because HLA antibodies can react with neutrophil assays and it cannot be determined whether the antibody is neutrophil specific or HLA related. When this occurs, an additional test with antigen capture assay is used. In this assay, monoclonal antibody immobilization of neutrophil antigens is done to differentiate between neutrophil antigen-specific antibodies from HLA antibodies [86, 87]. This assay can detect more than one neutrophil antigen-specific antibody.

A bone marrow study may be useful for patients with severe and prolonged neutropenia in whom isoimmune and maternal autoimmune neutropenia have been excluded. Bone marrow aspirate or biopsy may suggest the kinetic mechanism underlying the neutropenia by estimation of proliferative myeloid cells compared to postmitotic myeloid cells. For example, when neutropenia is caused by diminished neutrophil production, the proliferative compartment is decreased. In accelerated neutrophil usage or destruction, the proliferative compartment responds by expanding production. In addition, mature neutrophils are rapidly released into the peripheral blood, simulating a maturational arrest or decreased postmitotic pool. Despite its usefulness in narrowing the differential diagnosis, bone marrow studies almost never provide a precise diagnosis. For instance, no pathognomonic feature of bone marrow biopsy discriminates completely between hyporegenerative neutropenias in the neonatal period such as between neutropenia associated with maternal hypertension or Shwachman-Diamond syndrome. In a few instances, there are useful morphological clues in the bone marrow. For example, congenital neutropenia syndrome is suggested by enlargement and binucleation of promyelocytes and other myeloid precursors [84]. In autoimmune neutropenia, the bone marrow may contain macrophages with ingested antibody-coated neutrophils.

Neutrophil counts vary considerably early in the neonatal period with a mean of about 11,000 cells per microliter and a range between 6000 and 26,000 cells per microliter [3]. After the first 12 hours of life, neutrophil counts fall reaching a mean of 5000 cells per microliter (ranging from 1000 to 10,000 cells per microliter). Neutrophilia, defined as an elevation of the circulating absolute neutrophil count more than two standard deviations above the mean, may occur in response to both bacterial and viral infections, stress (post-operative or after seizures), or in conjunction with hemolytic anemia or immune thrombocytopenia. Occasionally, newborn infants exhibit an exaggerated response to infection with a significant increase in total WBC count (50,000 cells per

microliter) and increased early myeloid precursors in the peripheral blood, termed a leukemoid reaction [88]. The leukemoid reaction observed with a severe infection is often accompanied by cytoplasmic vacuoles within neutrophils and by the appearance of toxic granulations. The duration of the reaction ranges from days to weeks. Approximately 10% of infants with Down syndrome may have a transient leukemoid reaction that resembles congenital leukemia [85]. It is characterized by the presence of megakaryoblasts in the peripheral blood, hepatosplenomegaly, variable thrombocytopenia and infrequently hydrops fetalis and severe hepatic fibrosis. Abnormalities in all three hematopoietic lineages have been described. In most cases the leukemoid reaction is transient, although up to 30% of infants with Down syndrome infants who experience a neonatal leukemoid reaction develop acute megaloblastic leukemia later in childhood [85]. Leukaemoid reactions have also been reported in phenotypically normal infants who have trisomy 21 mosaicism associated with a clonal trisomy 21 in bone marrow cells [89, 90].

105.2.7 Prognosis

105.2.7.1 Infection

Infection remains a major cause of illness and death in the neonatal period [91, 92]. Newborn babies have an immature immune system and therefore may not show all signs of infection, and delayed treatment may lead to severe illness or death [93, 94]. Early treatment with antibiotics has been shown to reduce mortality due to sepsis in the neonatal period. Early treatment depends on a knowledge of risk factors and picking up early signs of infection [93, 94], which tend to be non-specific in this age group [95].

Although advances in neonatal intensive care have led to improved survival of very low birth weight (VLBW) and extremely premature infants, late onset sepsis (systemic infection after 48 hours of age) continues to be a significant cause of morbidity and mortality. The incidence of late onset sepsis increases with both decreasing birthweight and gestational age, and has been reported as occurring in approximately 25% of VLBW infants [96, 97]. Infants with the lowest birth weights are also more likely to have multiple episodes of sepsis [96]. In developing countries infection is estimated to cause 30–40% of neonatal deaths [98]. The spectrum of organisms responsible for early onset (vertically transmitted) sepsis differs from that associated with late onset (nosocomial) sepsis. This pattern becomes apparent from day two onwards [99]. Nosocomial infections are frequently associated with clinical deterioration including increased frequency of apnea or ventilatory requirements, temperature instability, abdominal distension, acidosis, lethargy, septic shock, necrotizing enterocolitis, meningitis and death [100]. The complications of necrotising enterocolitis and meningitis predispose an infant to an increased risk of future neurological impairment [101–103] and the mortality from late onset sepsis remains high, at 7–10% [96, 99]. This risk is secondary to immature immune responses, poorly developed skin and mucosal barriers to infection, numerous entry portals for organisms via cannulae, catheters and endotracheal tubes and continuing exposure to opportunistic organisms during a hospital stay, which is often prolonged.

105.2.7.2 Associated Abnormalities and Malignant Transformation

Congenital bone marrow disorders manifest by leukocyte defects are often associated with other congenital abnormalities. For instance, infants with Down syndrome may present with a variety of abnormalities in non-hematopoietic organ systems including cardiac and gastrointestinal malformations. Infants with Shwachman-Diamond syndrome exhibit exocrine pancreatic disorders and short stature. Barth syndrome is a mitochondrial disorder with associated development of cardiomyopathy and acidoisis. A predisposition to hematologic malignancy, in particular acute myelogenous leukemia (AML), is common among several of these disorders. It is postulated that the genetic disorder underlying these conditions provides a first hit expressed as variety of physical abnormalities, and a second hit later in life causes malignant transformation [104]. Downs syndrome, dyskeratosis congenita, Kostmann syndrome, and Shwachman-Diamond syndrome are among the syndromes associated with malignant transformation. Chronic granulomatous disease and cyclic neutropenia are not associated with malignant transformation.

105.2.8 Therapy and Treatment

105.2.8.1 Antibiotics

Early neonatal sepsis is mainly acquired from the mother. Vertical transmission of infection from mother to infant may take place before birth, during labor, or at the time of delivery. Most infants with peripartum acquired sepsis will develop clinical symptoms of sepsis within two days of life. After this period, nosocomial and community acquired infections start to play a bigger role.

The bacteria most commonly implicated in early neonatal sepsis are Group B *Streptococcus* and Gram-negative bacilli, and usually exclude coagulase negative *Staphylococcus*. Neonatal intensive care units or special care baby units tend to choose empirical first line antibiotic therapy that will cover both Gram-negative and Gram-positive bacteria. A combination of an aminoglycoside such as gentamicin and a beta-lactam such as penicillin has been the treatment of choice for early neonatal sepsis.

The range of organisms causing late onset sepsis includes gram positive and gram negative bacteria as well as fungal infection. As bacterial infections predominate, empiric antibiotic regimens focus on cover for both gram positive and negative bacterial infection. These antibiotics can be either narrow or broad spectrum in the range of organisms that they target. The epidemiology of late onset infection differs between developing and developed countries in the incidence of infection, the organisms responsible, and the subsequent mortality rates.

In general, prophylactic antibiotics are recommended for children with chemotherapy-induced neutropenia and children with chronic neutropenic conditions whose neutrophil count is less than 500 cells per microliter until recombinant G-CSF administration raises the neutrophil count to greater than 1000 cells per microliter [105, 106]. Broad use of prophylactic antibiotics for neutropenic neonates is not recommended because in most cases neutropenia is of limited duration and the general use of antibiotics may contribute to the emergence of bacteria with high antibiotic resistance. Individualization of care to include prophylactic antibiotics might be considered for an infant with severe neutropenia (500 cells per microliter) continuing for several days and requiring treatment with G-CSF to increase the neutrophil count above 1000 cells per microliter. Prophylactic antibiotics might also be considered for an infant with severe prolonged neutropenia who is unresponsive to G-CSF treatment. The precise antibiotics used in such cases should be selected based on the local nursery bacterial epidemiology.

105.2.8.2 Granulocyte Transfusions

The availability of new generations of effective antibiotics, recombinant hematopoietic growth factors to counter neutropenia, the risk of transmission of infection and the necessity for appropriate technology are some of the factors that have diminished the enthusiasm for use of granulocyte transfusions in neonates [107]. While there has been a resurgence of interest in enthusiasm for granulocyte transfusions in neutropenic patients, especially in patients with cancer, no recent studies have been conducted in newborn infants [108–112].

A recent systematic review evaluated the role of granulocyte transfusions as an adjunct to antibiotics in the treatment of neutropenic septic newborns [113]. This review included four eligible trials conducted on a small number of randomized infants [114–117].

There was no significant difference in mortality due to any cause during hospitalization in infants with sepsis and neutropenia who received granulocyte transfusions when compared with placebo or no granulocyte transfusion. In a single study by Cairo and coworkers, a reduction in all-cause mortality during hospital stay of borderline statistical significance, was observed when granulocyte transfusions were compared to intravenous immunoglobulin [117].

Granulocyte concentrates used for transfusion have potential adverse effects, including pulmonary complications, transmission of infections, fluid overload and graft versus host disease. Preparation of granulocytes for transfusion requires technical expertise and this is not universally available. Even among centers where it is available, there may be a delay in the procurement and transfusion of granulocytes after a decision to transfuse has been made. This delay may potentially render these transfusions of granulocytes less effective for neonates with neutropenia and sepsis.

105.2.8.3 IVIG

IVIG for Preventing Infection in Preterm and/or Low Birth Weight Infants

Administration of intravenous immunoglobulin provides IgG that can bind to cell surface receptors, provide opsonic activity, activate complement, and promote antibody dependent cytotoxicity. Intravenous immunoglobulin thus has the potential of preventing or altering the course of nosocomial infections. Ohlsson and Lacy conducted a systematic review to assess the effectiveness and safety of intravenous immunoglobulin (IVIG) administration compared to placebo or no intervention to preterm and/or low birth weight (LBW, less than 2500 gram birth weight) infants in preventing nosocomial infections [118]. Nineteen studies were included in this review, the most recent trial being in 2000. These included approximately 5000 preterm and/or LBW infants. Among qualifying studies the quantity of IVIG per dose varied widely from 120 mg/kg [119] to 1 g/kg [120]. Also, the number of doses varied from a single dose [119, 121–124] to seven doses [125]. Several different IVIG preparations were used. When all studies were combined the meta-analysis indicated that IVIG administration resulted in a 3% reduction in sepsis and a 4% reduction in any serious infection, one or more episodes, but was not associated with reductions in other important outcomes: sepsis, necrotizing enterocolitis, intraventricular hemorrhage, or length of hospital stay. Most importantly, IVIG administration did not have any significant effect on mortality from any cause or from infections. Prophylactic use of IVIG was not associated with any short-term serious side effects. From a clinical perspective the small reduction in nosocomial infections without a reduction in mortality or other important clinical outcomes is felt to be of marginal importance.

Antistaphylococcal Immunoglobulins to Prevent Staphylococcal Infection in Very Low Birth Weight Infants

Nosocomial infection continues to be a major problem affecting the immediate health and long-term outcome of preterm and very low birth weight neonates. More than half of these infections are caused by staphylococci. Various type specific

antibodies targeted at different antigenic markers of *Staphylococcus* have been developed and have shown promise in animal studies.

Shah and Kaufman conducted a systematic review to evaluate the efficacy and safety of anti-staphylococcal immunoglobulins in the prevention of Staphylococcal infection in very low birth weight infants [126]. Three eligible studies were included testing two different anti-staphylococcal immunoglobulin products. Two studies [127, 128] used pooled generic anti-staphylococcal immunoglobulin (INH-A21) and the third study [129] used antibody against type 5 and type 8 capsular polysaccharide antigen (Altastaph). These studies enrolled a total of 2,701 neonates. No significant differences were noted in the risk of staphylococcal infection or the risk of any infection between INH-A21 and placebo or Altastaph versus placebo. Furthermore, no significant differences were observed in the incidence of chronic lung disease, patent ductus arteriosus, necrotizing enterocolitis, intraventricular hemorrhage, retinopathy of prematurity or duration of antibiotic and vancomycin use. At the present time, anti-staphylococcal immunoglobulins (INH A-21 and Altastaph) are not recommended for prevention of staphylococcal infections in preterm or VLBW neonates. Further research to investigate the efficacy of other anti-staphylococcal products such as Pagibaximab under development may be forthcoming in the future.

IVIG for Treatment of Infections in Neonates

Ohlsson and Lacy conducted a systematic review to assess the effectiveness of intravenous immunoglobulin (IVIG) in reducing mortality and morbidity caused by suspected and proven infection in newborn infants [130]. Five hundred fifty three neonates with suspected infection were enrolled in randomized clinical trials in seven countries to evaluate the effect of IVIG on neonatal outcomes [131–138]. Six studies enrolling 318 infants reported on the outcome of mortality for randomized patients with clinically suspected infection. Results of the meta-analysis showed a reduction in mortality following IVIG treatment of borderline statistical significance. Treatment with IVIG (seven trials, n = 262) in cases of subsequently proven infection resulted in statistically significant reduction in mortality. There is insufficient evidence to support the routine administration of IVIG preparations investigated to prevent mortality in infants with suspected or subsequently proved neonatal infection. Moreover, well-designed trials will be required to confirm or refute the effectiveness of IVIG to reduce adverse outcomes in neonates with suspected infection.

105.2.8.4 G-CSF and GM-CSF

In the United States, G-CSF is approved for use by the Food and Drug Administration for use in patients with severe chronic neutropenia, cancer patients receiving myelo-suppresive chemotherapy, cancer patients receiving bone marrow transplantation and patients undergoing peripheral blood hematopoietic stem cell collection. Patients with severe chronic neutropenia generally derive considerable benefit from G-CSF administration. Varieties of neutropenia in neonates for which G-CSF treatment is effective are Kostmann syndrome, Shwachman-Diamond syndrome, cyclic neutropenia, and alloimmune neutropenia.

105.2.8.5 Bone Marrow and Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation remains the only available treatment for patients with congenital neutropenia refractory to G-CSF treatment. Absence of response to G-CSF, G-CSF receptor mutation, and leukemic transformation are absolute indications for hematopoietic stem cell transplantation if a suitable donor is available. Pulmonary mycosis and pulmonary abscesses do not represent absolute contraindications to bone marrow transplantation, although a relapse rate of 30–50% for mycosis has been reported, despite adequate medical and surgical treatment [139, 140].

G-CSF has had a major impact on the management of severe congenital neutropenia, cyclic neutropenia, and Shwachman-Diamond syndrome. Almost all patients respond to G-CSF with increased neutrophils, reduced infections, and improved survival. Some responders with congenital neutropenia and Shwachman-Diamond syndrome have developed myelodysplastic syndrome and acute myeloid leukemia, which raises the question of the role of G-CSF in pathogenesis. The issue is complicated because both disorders have a propensity for myelodysplastic syndrome or acute myeloid leukemia as part of their natural history. To address this, the Severe Chronic Neutropenia International Registry used its large database of chronic neutropenia patients treated with G-CSF to determine the incidence of malignant myeloid transformation in the two disorders, and its relationship to treatment and to other patient characteristics. No statistically significant relationships were found between age at onset of myelodysplastic syndrome or acute myeloid leukaemia and patient gender, G-CSF dose, or duration of G-CSF therapy. What was observed, however, was the multistep acquisition of aberrant cellular genetic changes in marrow cells from patients who transformed, including activating ras oncogene mutations, clonal cytogenetic abnormalities, and G-CSF receptor mutations.

In murine models, G-CSF receptor mutation produces a hyperproliferative response to G-CSF, confers resistance to apoptosis, and enhances cell survival. Since congenital neutropenia and Shwachman-Diamond syndrome are inherited forms of bone marrow failure, G-CSF may accelerate the propensity for myelodysplastic syndrome and acute myeloid leukaemia in the genetically altered stem and progenitor cells,

especially in those with G-CSF receptor and ras mutations (82% and 50% of patients who transform, respectively). Alternatively, and equally plausible, G-CSF may simply correct neutropenia, prolong patient survival, and thus allows time for the malignant predisposition to declare itself. In patients who transform to overt myelodysplastic syndrome or acute myeloid leukemia, hematopoietic stem cell transplantation is the only chance for cure. In those with an isolated clonal cytogenetic change, but without other evidence of myelodysplastic syndrome, or with an isolated G-CSF receptor mutation, there is room for conservative management. One option is to reduce the G-CSF dosage as much as possible, and observe progression, if any, to more overt signs of malignancy [141].

Reticular dysgenesis is a very rare congenital immunodeficiency classified within the severe combined immunodeficiencies and characterized by impairment of both lymphoid and myeloid cell development. Bertrand and colleagues reported 10 patients with reticular dysgenesis, treated with HLA-haploidentical hematopoietic stem cell transplantation. All children but one were symptomatic within the first days of their lives. Five patients required two hematopoietic stem cell transplants. Five patients received conditioning therapy with busulfan and cyclophosphamide. Three of them survived with myeloid and T- and B-cell lymphoid reconstitution, whereas two patients died (one chronic graft-versus-host disease, one pneumonitis). Transplantation without or with other conditioning regimens in the other five cases led to absent or incomplete engraftment and none of these cases survived. These results demonstrated the importance of intensive conditioning before haploidentical hematopoietic stem cell transplantation in reticular dysgenesis to achieve full lymphoid and myeloid engraftment [54, 56].

Curently allogenic hemopoietic stem cell transplant is the only curative treatment available for severe congenital neutropenia, leukocyte adhesion deficiency, and chronic granulomatous disease [142].

105.2.8.6 Gene Therapy

Chronic granulomatous disease is a rare congenital disorder resulting from a failure of neutrophils to produce oxidases. Patients are therefore prone to recurrent infections from various organisms including fungi and atypical bacteria. The mortality in patients with the X-linked form of chronic granulomatous disease, the most common type, ranges from 3% to 5% per year and although management of infections has improved with advances in antimicrobial therapies, better methods are needed to cure these patients. Peripheral blood stem cell or bone marrow transplantation, while curative, is not widely used due to the episodic nature of the infections and the belief by many that conservative management is preferable to the risks of transplantation [143]. Still, as will be discussed, improvements in the field are making allogenic transplantation more desirable and tilting the risk benefit ratio in favor of this modality. Additionally, gene therapy, which has been considered a possible method to cure chronic granulomatous disease, has within the last 5–10 years become more and more of a reality and may be realized in the not to distant future [144, 145].

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Neonatal Hereditary Neutropenia

Gaetano Chirico and Carmelita D'Ippolito

106.1 Introduction

Hereditary neutropenia includes many disorders of distinct origin and variable prognosis, characterized by a reduction of the absolute neutrophil count (ANC) that predisposes patients to bacterial infections, in particular pyogenic infections, such as cutaneous cellulitis, deep abscesses, pneumonia and sepsis [1, 2]. Susceptibility to bacterial infections, even in patients with severe neutropenia, can be quite variable, depending on the underlying etiology. Congenital neutropenia my be associated with extraematopoietic manifestations.

106.2 Severe Congenital Neutropenia (SCN)

In 1956 Kostmann described congenital neutropenia as an autosomal recessive disease in a large intermarried Swedish family. The incidence is estimated to be approximately 2 cases per million of the population [1]. Affected patients regularly have episodes of fever, skin infections, stomatitis, pneumonia and perirectal abscesses that usually begin in the first months of life and lead to death during infancy and childhood. The ANC is less than 0.2×10^9 cells/L. Eosinophylia, monocytosis and splenomegaly may be present.

The bone marrow examination reveals the typical arrest of the myeloid cell differentiation at the promyelocytic stage with a marked depletion of mature neutrophils [1, 3].

Recent studies on the genetic basis of SCN have detected mutation in the leucocyte elastase gene, *ELA2*, a gene defect that is inherited as an autosomal dominant trait in about 60–80% of patients [4], a gene defect that is inherited as an autosomal dominant trait. *ELA2* encodes a serine protease synthesized during the promyelocyte/myelocyte stage, which is stored in the primary granules [4]. Correct localization of the neutrophil elastase (NE) in the primary granules requires interaction of NE with the adaptor protein complex AP3 that shuttles transmembrane cargo proteins from the Golgi network to the lysosomes. Most mutations in *ELA2* remove the tyrosine-based recognition sequence for the AP3μ subunit, thereby favoring the misallocation of the enzyme to the membrane instead of inside the granules. It was proposed that mislocalized NE must drastically reduce the production of neutrophils from the myeloid progenitor cells in favor of monocytes.

The introduction of hematopoietic growth factors has greatly improved both life span and quality of life [5]. However, patients with SCN have a heterogeneous response to G-CSF therapy and often require a gradual increase of the dosage that generally ranges from 11 to 13 μg/kg/die. In addition 3–5% of SCN patients are refractory to G-CSF treatment.

Finally a significant number of children who are receiving G-CSF therapy are developing myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), the overall risk being between 9 and 13% [3]. The exact relationship between G-CSF treatment and the risk of leukemic transformation is still unclear [5]. A subset of SCN patients, namely those with more severe disease, are more prone to develop MDS, AML or both, probably because of exposure to other leukemogenic factors such as monosomy of chromosome 7, alterations of chromosome 21, activating mutations of the oncogene *ras*, and mutation in the G-CSF receptor (*CSF3R*) [5].

In the light of this finding G-CSF treatment seams a safe option for the vast majority of patients with neutropenia, while patients at risk for leukemic transformation would benefit from hematopoietic stem cell transplantation.

106.3 Cyclic Neutropenia

Cyclic neutropenia is an autosomal dominant or sporadic condition, characterized by periodic episodes of severe neutropenia with a nadir of less than 0.2×10^9 cells/L, that occur usually every 21 days [6].

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The neutropenic periods persist for 3–5 days and are accompanied by malaise, fever, gingivitis, oral ulcers and lymphoadenopathy. There are also regular oscillations of the other leukocyte subsets, reticulocytes and platelets. Symptoms begin during the first year of life, are often milder after puberty and are usually less severe than in SCN [3, 4, 6] even though fatal *Clostridium* bacteremia has been reported in untreated patients [3]. A variety of studies suggest that cyclic neutropenia occurs because of a periodic disruption in cell production in the bone marrow [3]. And it has recently been shown that a heterozygous mutation of *ELA2* is the genetic cause of the disease. The mutations reported in cyclic neutropenia are located in the same gene associated with SCN, but in different exons resulting in preferential accumulation of NE in granules – an example of genotype/phenotype correlation.

In the management of those patients the use of prophylactic G-CSF treatment is recommended, it has been very effective in improving peripheral blood neutrophil counts and avoids symptoms and infections [5]. The dose of G-CSF required is usually lower than in SNC: 2–3 μg/kg/die or on alternate days, the occurrence of MDS or leukemia has not been reported in patients with cyclic neutropenia [5].

106.4 Myelocathexis and WHIM Syndrome

Myelocathexis is a rare autosomal dominant disorder characterized by moderate to severe chronic neutropenia resulting from the retention of mature neutrophils in the bone marrow.

Myelocathexis is often associated with hypogammaglobulinemia, leucopenia and warts, a clinical picture recognized as WHIM syndrome (warts, hypogammaglobulinemia, infections, myelokathexis) [7], an autosomal dominant disease.

In WHIM syndrome ANCs are usually $<0.5\times10^9$/L, but during infectious episodes their number rises suddenly, allowing a more benign course with recurrent mild respiratory infections. Early death related to infections has not been reported. Recent studies showed that WHIM syndrome is caused by heterozygous mutations in the gene coding for the chemokine receptor CXCR4. Cells expressing the mutated CXCR4 have an increased responsiveness to chemokines and thus are not released from the bone marrow to the circulating blood pool [7]. The neutropenia associated with myelocathexis and WHIM is only partially corrected by administration of G-CSF [8].

106.5 Congenital Neutropenia with Extra Hematopoietic Manifestations

106.5.1 Hermansky-Pudlak Syndrome 2

This autosomal recessive disease is characterized by neutropenia, oculocutaneous albinism and moderate bleeding disorders [9]. It is caused by mutations of the gene encoding for the beta3 component of the AP3 complex, again preventing the transport of NE (and other proteins) from the Golgi network to the lysosomes in hematopoietic cells and in melanocytes [9].

The severe neutropenia is responsive to G-CSF treatment.

106.5.2 Shwachman-Diamond Syndrome

The Shwachman-Diamond syndrome (SDS) is a rare multiorgan disease inherited as an autosomal recessive trait that combines neutropenia, exocrine pancreatic insufficiency, skeletal abnormalities and short stature [10].

The symptoms begin early in infancy with bacterical infections (pneumonia, otitis media, osteomyelitis, skin infections, sepsis) and failure to thrive because of intestinal malabsorption.

Chronic neutropenia is constantly observed in all patients and two thirds of them have a neutrophil count less than 1×10^9 cells/L, so while the neutropenia can be intermittent it is never cyclic. Mild anemia and thrombocytopenia are described commonly [10].

Cytopenia reflects hematopoietic dysplasia, which, in association with cytogenetic abnormalities, may increase the risk of transformation in MDS/AML mainly in older children. For this reason annual bone marrow aspirates are recommended in patients with SDS. Indeed follow-up studies of SDS patients demonstrate that some cytogenetic changes my spontaneously regress.

The causative gene of the disease has recently been identified and was named *SDBS*. It is expressed in both hematopoietic and non hematopoietic tissues. Treatment of patients suffering from SDS includes pancreatic enzyme replacement and administration of G-CSF, which increases ANC to normal levels and should be started in case of severe infections [11].

106.5.3 Neutropenia Associated with Glucose-6-Phosphatase Complex Disorders

Glucose 6 phosphatase is a complex of three proteins bound to the endoplasmic reticulum responsible for glycogenolysis. Two of these three proteins are associated with congenital neutropenia: the translocase (SLC37A4), and G6PC3 that is a catalytic protein.

The association between these molecular changes and neutropenia is not clear because the glycogenolysis pathway is not the source of energy normally used by neutrophils, which mainly use the pentose pathway; this raises the hypothesis that this protein has another functions in neutrophils.

106.5.3.1 Glycogen Storage Disease Type Ib

It is a metabolic disorder characterized hepatic glycogen accumulation, intolerance of fasting, hypoglycemic events, and hyperlactacidemia, as well as susceptibility to infections [12] and colitis resembling Crohn's.

This susceptibility to infections is due to neutropenia and, sometimes, to neutrophil dysfunction (defective chemotactism). The origin of the neutropenia and neutrophil dysfunction is unknown.

106.5.3.2 G6PC3 Mutations

G6PC3 mutation prevailing in Armenia is caracterized by severe permanent neutropenia with granulocyte maturation arrest, susceptibility to infections, and several other clinical manifestations, (thin skin, urogenital malformations, and cardiac disorders). Mutations of the G6PC3 gene are generally homozygous, but a double heterozygote has been described [13].

106.5.4 Neutropenia Associated with Poikilodermia, Clericuzio Type

Clericuzio type neutropenia with genodermatosis that onset in the first year of life. Recurrent infections occur, and especially pneumonia. The neutropenia is often severe. The poikilodermia includes skin atrophy and popular erythematous rash. Composite mutations of the C16ORF57 gene are responsible of pathology [14].

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Therapy with Recombinant Leukocyte Growth Factors

Robert D. Christensen

107.1 Discovering Neutropenia in a Neonate

The complete blood count (CBC) is one of the most commonly obtained laboratory tests in Neonatology. When a neonatologist finds a low blood concentration of neutrophils, it can be expected as in a neonate with septic shock, or it can be unanticipated and puzzling. A neonatologist finding neutropenia might wonder: What conditions should I consider in the differential diagnosis? Will the neutropenia be a significant clinical problem or will it be trivial and transient? Should I order rG-CSF? This chapter will focus on the last of these questions, reviewing the studies and the reasoning involved in this decision, and will include an algorithm as a guide to answering this question.

Preparatory to that discussion, it is imperative to first define neutropenia. Consider the number of neutrophils per microliter of blood, also known as the ANC (absolute neutrophil count) and not just the WBC (white blood cells or leukocytes per microliter). If the ANC is less than 1000/μL the neonate has neutropenia. If the ANC is < less than < 500/μL the patient can be said to have severe neutropenia [1–3]. Counts above 1000/μL can technically be neutropenic, if they fall below the 5th% reference range.

For instance, as shown in Fig. 107.1 an ANC of 3000/μL 12 hours after birth is indeed an abnormally low ANC [4, 5]. The fact that the ANC is below the reference range signals the presence of pathology. However, it is doubtful that an ANC as high as 3000/μL constitutes a host-defense deficiency, or renders the patient at high risk for acquiring an infection. Therefore we recommend using the diagnosis neutropenia, or placing neutropenia on the problem list, only if the ANC is less than 1000/μL.

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107.2 The Importance of Neutrophils in Neonatal Host Defense

Neutrophils are pivotal to the process of antibacterial defense [6, 7], and patients lacking neutrophils can experience repeated local and systemic infections [8, 9]. Severe chronic neutropenia (SCN) is a cluster of diagnoses bearing the common feature of neutropenia, generally severe neutropenia, present from birth [10, 11].

The advent of recombinant granulocyte colony-stimulating factor (rG-CSF) dramatically improved the lives of patients with SCN, elevating their circulating neutrophil concentrations, markedly reducing infectious illnesses, and extending their life expectancy [8, 12].

SCN can be diagnosed in a neonate [13, 14]. However, the majority of patients with SCN are not diagnosed until they are several months old, following many infectious episodes that prompted evaluations into immunological deficiencies. When SCN is diagnosed in a neonate, that patient should receive the benefit of rG-CSF treatment [8, 12–15]. When a transient variety of neonatal neutropenia is diagnosed, distinct from SCN, the benefit of rG-CSF treatment is speculative and unproven [16–20]. This chapter will review the biological plausibility, the animal studies, and the clinical trials aimed at testing rG-CSF and rGM-CSF treatment for neonates with neutropenia.

107.3 Leukocyte Growth Factors in Severe Chronic Neonatal Neutropenia

107.3.1 Kostmann Syndrome

Table 107.1 lists varieties of neutropenia that are generally considered as part of the SCN syndrome. The condition is the result of mutations in the *ELA2* (neutrophil elastase) gene [24–27]. Although rG-CSF treatment is effective in increasing blood neutrophils and reducing febrile illnesses, it does not

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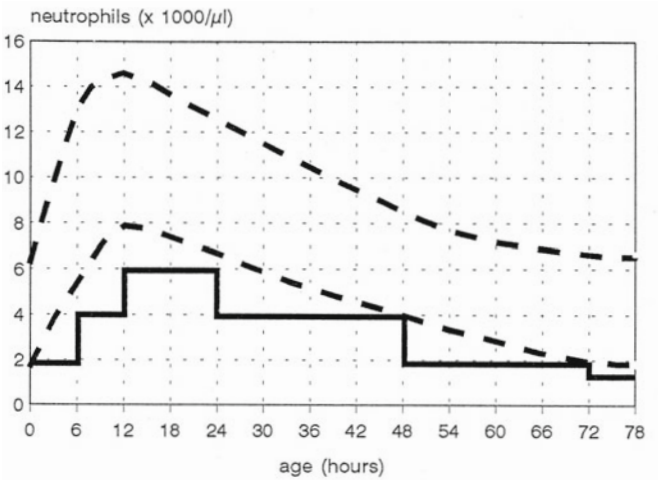
Birthweight > 1500 g

Age (hours)	Neutropenia
0–6	< 2000/μL
>6–12	< 4000/μL
>12–24	< 6000/μL
>24–48	< 4000/μL
>48–72	< 2000/μL
>72	< 1500/μL



Birthweight ≤ 1500 g

Age (hours)	Neutropenia
0–6	< 500/μL
>6–12	< 1500/μL
>12–30	< 1800/μL
>30–48	< 1500/μL
>48	< 1100/μL

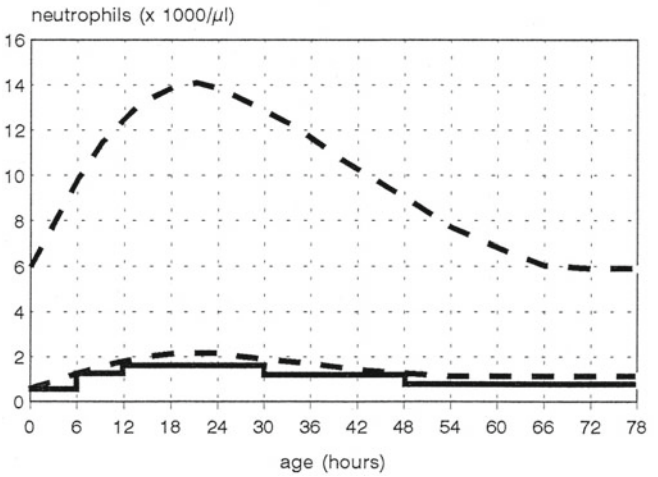


Fig. 107.1 Definitions of neutropenia

Table 107.1 Varieties of neutropenia among neonates that are generally considered SCN

Kostmann syndrome
Shwachman-Diamond syndrome
Barth syndrome
Cartilage-hair hypoplasia
Cyclic neutropenia
Glycogen storage disease type 1b
Severe neonatal immune-mediated neutropenias

generally correct the gingivitis that is a prominent feature of this condition in some families. This is probably because rG-CSF does not increase the natural antimicrobial peptide (LL-37) deficiency in these patients [28, 29].

107.3.2 Shwachman-Diamond Syndrome

This variety of SCN is generally diagnosed after manifestations of exocrine pancreatic insufficiency, with diarrhea and failure to thrive. It is generally an autosomal recessive condition. Some children with this syndrome respond favorably to rG-CSF, yet some progress to bone marrow failure and require marrow transplantation [30, 31].

107.3.3 Barth Syndrome

These patients are generally males (X-linked) with dilated cardiomyopathy, organic aciduria, growth failure, muscle weakness, and neutropenia [32]. rG-CSF can be helpful in these patients as an adjunct to treating infections, or as a preventive measure if their neutropenia is sufficiently severe [33, 34].

107.3.4 Cartilage-Hair Hypoplasia

This is a form of short-limbed dwarfism associated with frequent infections. These patients have short pudgy hands, redundant skin, and hyperextensible joints in the hands and feet but flexor contractions at the elbow. Neutropenia occurs in some patients with cartilage-hair hypoplasia and these can benefit from rG-CSF administration [35].

107.3.5 Cyclic Neutropenia

This condition is caused by mutation in the *ELA2* (neutrophil elastase) gene, and results in periodic drops in blood neutrophil concentration, generally on a three to four-week cycle [36, 37]. Counts can drop to < 500/μL or lower, and infections can be a periodic problem. Because it generally takes several cycles before the diagnosis is considered, most cases are not diagnosed as neonates. rG-CSF administration is useful in preventing the very low nadir counts and thereby preventing infectious complications [36–38].

107.3.6 Glycogen Storage Disease Type 1b

Von Gierke disease is an autosomal recessive disorder caused by a deficiency of the enzyme glucose-6-phosphate translocase,

which transports glucose-6-phosphate into the endoplasmic reticulum for further metabolism. In GSD-1b, glucose-6-phosphate accumulates intracellularly. Affected neonates present with hypoglycemia, hepatomegaly, growth failure and neutropenia. Patients with GSD-1b have recurrent bacterial infections, oral ulcers, and inflammatory bowel disease. The gene causing GSD-1b is located on chromosome 11q23 [39]. rG-CSF can help these patients avoid the recurrent bacterial infections that are otherwise a problematic part of the condition.

107.3.7 Severe Immune-Mediated Neonatal Neutropenia

Most of the severe and prolonged immune mediated neonatal neutropenias are alloimmune [40–43]. However, a few severe and prolonged cases have been found to be autoimmune (maternal autoimmune disease) [43, 44], and a few have been found to be autoimmune neutropenia of infancy (a primary isolated autoimmune phenomenon in neonates) [43, 45].

Alloimmune neonatal neutropenia is a relatively common condition where the mother develops antibodies to antigens present on paternal and fetal neutrophils [40–44]. Antineutrophil antibodies have been found in the serum of as many as 20% of randomly surveyed pregnant and postpartum women [43, 44]. Mostly, such antibodies cause little problem to the fetus and neonate, but up to 2% of consecutively sampled neonates have neutropenia on this basis. This variety of neutropenia can be severe and prolonged, with a median duration of neutropenia of about seven weeks, but a range up to six months. Repeated infections can occur in these patients until their severe neutropenia remits. Delayed separation of the umbilical cord and skin infections are the most common infectious complications, but serious and life-threatening infections can occur. The mortality rate in this condition, due to overwhelming infection, is reported to be 5%. Severe cases have been successfully treated with rG-CSF. Unlike patients with other varieties of SCN, the neutropenia in this condition will remit spontaneously and the rG-CSF treatment can be stopped. Remission occurs when maternal antineutrophil antibody in the neonate has dropped significantly.

Neonatal autoimmune neutropenia occurs when mothers have autoimmune diseases, and their antineutrophil antibodies cross the placenta and bind to fetal neutrophils. Clinical features are generally milder than in alloimmune neonatal neutropenia and it is rare that a neonate with this variety of neutropenia needs rG-CSF treatment.

Autoimmune neutropenia of infancy is an unusual disorder where the fetus, and subsequently the neonate, has a primary isolated autoimmune phenomenon [46–50]. Neutrophil specific antibodies are found in the neonate's serum, reactive against his/her own neutrophils, but no antibodies are found in the mother's serum. Most cases occur in children between three and 30 months of age, with a reported incidence of 1:100,000 children. Affected children most often present with minor infections. Bux reported 240 cases and reported that 12% presented with severe infections, including pneumonia, sepsis, or meningitis [48]. The neutropenia in this condition generally persists much longer than in cases of alloimmune neutropenia, with a median duration of about 30 months and a range from 6–60 months [49, 50]. This variety of neutropenia can be severe, with blood neutrophil concentrations often < 500/μL. rG-CSF administration can increase the neutrophil count and reduce infections complications [48, 50].

107.4 Leukocyte Growth Factors in Neonatal Neutropenia not Categorized as SCN

107.4.1 Pregnancy Induced Hypertension

This is the most common variety of neutropenia seen in the NICU [51–58]. Perhaps 50% of neonates born to mothers with PIH have this variety of neutropenia. The ANC can be very low, frequently < 500/μL, but the count generally rises spontaneously within the first days, and is almost always > 1000/μL by day three. Usually no leukocyte left shift is seen, and no toxic granulation, Dohle bodies, or vacuolization are present in the neutrophils. It is not clear whether this variety of neutropenia predisposes neonates to acquire bacterial infection. Usually the condition is so transient that such a predisposition is unlikely. The condition is probably caused by an inhibitor of neutrophil production of placental origin that might function mechanistically by depressing natural G-CSF production [51–53].

107.4.2 Severe Intrauterine Growth Restriction

This variety of neonatal neutropenia seems to be identical to that associated with PIH. We observed no difference in the onset, duration, or severity of neutropenia in SGA neonates versus neonates born after PIH [59]. Obviously, some neonates born after PIH are also SGA, and it might be that the

Table 107.2 Varieties of neutropenia among neonates that are NOT classified as SCN

Pregnancy induced hypertension
Severe intrauterine growth restriction
The twin-twin transfusion syndrome
Rh hemolytic disease
Bacterial infection
Necrotizing enterocolitis
Chronic idiopathic neutropenia of prematurity

most severe neutropenias in this category are among those with both PIH and SGA. We assume that the neutropenia of PIH and SGA are mechanistically similar and that both are transient with few clinical consequences and no need for rG-CSF administration.

107.4.3 The Twin-Twin Transfusion Syndrome

The donor in a twin-twin transfusion is generally neutropenic, but the recipient can also have neutropenia, although usually not as severe [60]. As with the varieties of neutropenia accompanying PIH and SGA, there is generally no leukocyte left shift nor are there neutrophil morphological abnormalities. This condition is also transient, with the ANC generally spontaneously rising to >1000/μL by 2–3 days, and thus no rG-CSF administration is warranted.

107.4.4 Rh Hemolytic Disease

Neonates with anemia from Rh hemolytic disease are almost always neutropenic on the first day of life [61]. This variety of neutopenia is similar to that of PIH/SGA and donors in a twin-twin transfusion, and is likely due to reduced neutrophil production. The neutropenia is transient, generally resolving in a day or two, and thus no specific treatment is generally required for the neutropenia.

107.4.5 Bacterial Infection

Two strategies have been proposed for rG-CSF usage during neonatal infections.

Since neutropenia commonly accompanies overwhelming septic shock in neonates, perhaps rG-CSF might be a reasonable adjunct to antibiotics and intensive care treatment. Second, since neutrophil function, particularly chemotaxis, is immature among neonates, perhaps rG-CSF administration might be a reasonable way to prevent nosocomial infections among high-risk neonatal patients.

Animal models for both potential uses of rG-CSF were established and supported these hypotheses. In a Cochrane review, Carr et al examined both potential uses [62]. They located seven studies where infected neonates were treated with rG-CSF versus placebo [62–68] and three studies where rG-CSF versus placebo was used as prophylaxis against infections [69–70]. They found no evidence that the addition of rG-CSF or rGM-CSF to antibiotic therapy in preterm infants with suspected systemic infection reduces immediate all-cause mortality. No significant survival advantage was seen at 14 days from the start of therapy [typical RR 0.71 (95% CI 0.38, 1.33)]. They conducted a subgroup analysis of 97 infants from three of the studies who, in addition to systemic infection, had a low neutrophil count (<1700/μL) at trial entry. This subgroup did show a significant reduction in mortality by day 14 [RR 0.34 (95% CI 0.12, 0.92); RD –0.18 (95% CI –0.33, –0.03); NNT 6 (95% CI 3–33)].

The three prophylaxis studies [70–72] did not show a significant reduction in mortality in neonates receiving rGM-CSF [RR 0.59 (95% CI 0.24, 1.44); RD –0.03 (95% CI –0.08, 0.02)]. The identification of sepsis as the primary outcome of prophylaxis studies has been hampered by inadequately stringent definitions of systemic infection. However, data from one study suggest that prophylactic rGM-CSF might provide protection against infection when given to preterm infants who are neutropenic [72].

Carr et al concluded that there is currently insufficient evidence to support the introduction of either rG-CSF or rGM-CSF into neonatal practice, either as treatment of established systemic infection to reduce resulting mortality, or as prophylaxis to prevent systemic infection in high-risk neonates. This conclusion is consistent with other meta-analyses and reviews [71–83].

107.4.6 Necrotizing Enterocolitis

Neutropenia is relatively common among severe cases of NEC. In some cases the neutropenia is transient and resembles the neutropenia following endotoxin [84, 85]. No studies have focused on using rG-CSF among neutropenic neonates with NEC.

107.4.7 Chronic Idiopathic Neutropenia of Prematurity

Certain preterm neonates develop neutropenia when 4–10 weeks old. This variety of netropenia is often associated with a patient's spontaneous recovery from the anemia of prematurity. Neutrophil counts are generally <1000/μL but rarely <500/μL [86–89]. The condition is transient, lasting a few weeks to perhaps a month or more. It appears to be a hyporegenerative neutropenia, because it is not accompanied by a leukocyte left shift nor morphological abnormalities of the neutrophils.

Patients with this condition have an "rG-CSF mobalizable neutrophil reserve", meaning that if rG-CSF is given, their neutrophil count increases within hours. This has been taken as evidence that these patients do not have a significant host-defense deficiency and they can supply neutrophils to tissues when needed [87]. Thus, although these patients can be neutropenic for several weeks, this condition is likely benign and needs no treatment.

107.5 The Use of Leukocyte Growth Factors

G-CSF and GM-CSF are naturally occurring proteins involved in proliferation and differentiation of myeloid precursors into mature neutrophils [90]. The genes for both have been cloned, and the purified recombinant factors are commercially available in pharmacologic quantities [91]. These factors have been widely used in adult and pediatric medicine primarily to treat iatrogenic neutropenia and bone marrow failure syndromes [91, 92]. They can shorten the duration of neutropenia after chemotherapy for leukemia and solid tumors and after bone marrow transplantation. G-CSF is used in patients undergoing peripheral blood hematopoietic stem cell collection. Target cells for these factors differ. G-CSF is lineage specific for the committed progenitors of neutrophils [93, 94]. It stimulates proliferation and differentiation, expanding the available pool of neutrophil precursors and shortening their transit time through the marrow. It has been reported that G-CSF enhances several functions of mature neutrophils, but this has been questioned [95]. GM-CSF acts on multilineage progenitors and on those of monocyte and neutrophil lineage and enhances bactericidal activities of mature phagocytes [90, 91].

Patients with SCN generally derive considerable benefit from rG-CSF administration. Almost all respond to doses of 5–10 micrograms per kg administered at intervals ranging from every day to once per week in order to achieve neutrophil concentrations above 500–1000/μL [90–94].

Explanted blood monocytes and mononuclear cells from human neonates produce G-CSF and GM-CSF poorly, compared with cells from adults. Moreover, cells from preterm infants produce these proteins more poorly than do cells from term infants [95, 96]. Plasma concentrations of G-CSF are relatively low in newborn infants with neutropenia and in infants with presumed sepsis [97, 98]. Neonatal hematopoietic progenitor cells are equally responsive as adult bone marrow progenitors to the actions of G-CSF and GM-CSF in culture. The rapid neutrophil response to infection is impaired in the murine model of G-CSF gene disruption [98]. Animal studies demonstrated diminished production of G-CSF by neonates compared with adults during bacterial sepsis [100, 101]. rG-CSF and rGM-CSF administration in animal models of neonatal sepsis have documented improved survival [100–102].

The decision regarding whether to administer rG-CSF to a neutropenic infant must consider risks and benefits. Animal studies suggesting benefits administered the hematopoietic growth factor concurrently or within hours of inoculation with the bacterial agent. Under the best of circumstances treatment of clinical sepsis with rG-CSF or rGM-CSF within hours of bacterial invasion may not be feasible. In some infants with overwhelming sepsis bone marrow neutrophil storage pools may already be depleted, thus limiting the capability for response to rG-CSF or rGM-CSF treatment. Additionally, septic and neutropenic infants exhibit elevated circulating and urinary G-CSF concentrations suggesting that their G-CSF receptors are saturated with endogenous G-CSF [103].

The risk of developing nosocomial infection in preterm neonates with neutropenia due to maternal hypertension is controversial [104]. Some studies have demonstrated an increased incidence of nosocomial infections in these neonates, but others have not. Studies evaluating rG-CSF administration to correct the neutropenia caused by maternal hypertension have demonstrated a significant increase in circulating neutrophils compared with study entry, but have not demonstrated lower rates of infection [105, 106].

rGM-CSF has been studied in neonates using slightly different strategies than used in trials of rG-CSF administration. Three prophylaxis studies using rGM-CSF have been published. These studies adopted different treatment regimens and did not use comparable criteria for identifying episodes of systemic infection. rGM-CSF increased the circulating absolute neutrophil count and bone marrow neutrophil storage pool. Neutrophil function was enhanced as defined by up-regulation of surface C3bi expression [70, 71]. Circulating monocytes and platelet counts were increased among rGM-CSF recipients. A systematic review of these studies concluded that rGM-CSF prophylaxis did not lead to a significant reduction in mortality [62].

Similarly, a recent multicenter randomized controlled trial of prophylactic rGM-CSF for extremely preterm infants at high risk of sepsis did not demonstrate reductions in sepsis or improved survival [107].

107.6 A Consistent Approach to the Use of rG-CSF and rGM-CSF in the NICU

Fig. 107.2 is a simple algorithm for making the decision regarding rG-CSF administration in the NICU. It is intended as a guideline to serve until sufficient data accumulate for an evidence-based change in this approach. The algorithm is in

Table 107.3 Screening for severe chronic neutropenia

Inclusion questions:

1. Has a blood neutrophil count of <500/μL been documented on at least three occasions in the past three months?
2. Is there a history of recurrent infections? (specify)
3. Is the bone marrow evaluation consistent with severe chronic neutropenia? (date performed)
4. Has a cytogenetic evaluation been completed?
5. Is the patient now receiving Neupogen (rG-CSF)?

Exclusion criteria

1. The neutropenia is known to be drug-induced
2. Thrombocytopenia is present (<50,000/μL) except in the case of Shwachman-Diamond syndrome or glycogen storage disease type 1b
3. Anemia is present (hgb <8g/dL) except in the case of Shwachman-Diamond syndrome or glycogen storage disease type 1b
4. The patient has a myelodysplastic syndrome, aplastic anemia, is HIV positive, has some other hematological disease, has rheumatoid arthritis, or has had previous chemotherapy for cancer

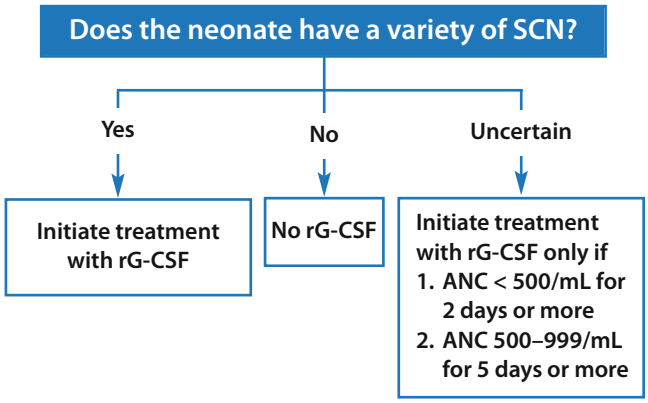


Fig. 107.2 The US FDA approved rG-CSF for use in children with severe chronic neutropenia, and for children receiving myelosuppressive chemotherapy of marrow transplantation. The rG-CSF used in the USA is produced by Amgen (Thousand Oaks, CA) and has a molecular weight slightly lower than natural G-CSF, because it is produced in *Eschericha coli* and is not glycosylated. It has an amino acid sequence identical to that of natural G-CSF except for the addition of an N-terminal methionine, which is necessary for its expression in *E. coli*. SCN includes Kostmann syndrome, Shwachman-Diamond syndrome, cyclic neutropenia, Bart syndrome, autoimmune or alloimmune neonatal neutropenia, or any syndrome or condition where severe and chronic neutropenia are a consistent finding. G-CSF administration is not recommended for neonates with neutropenia of a non-severe, or a non-chronic nature, such as the neutropenia associated with PIH, sepsis, or chronic idiopathic (mild) neutropenia

keeping with FDA approved approaches and avoids off-label uses. The principal question is whether the neutropenic neonate has a variety of SCN; a question to which there are three possible answers; 1) Yes, 2) No and 3) Uncertain. We propose that if the answer is yes, the patient should be afforded the benefit of rG-CSF treatment, and if the answer is no, rG-CSF should not be given. Cases where the answer is uncertain, might receive rG-CSF if the neutropenia is severe (ANC <500/μL for 2 days of more) or prolonged (<1000/μL for 5 days or more) while the question of SCN is being actively investigated.

An international registry for SCN cases can be accessed at the website http://depts.washington.edu/registry/, using the entry criteria and exclusion criteria given in Table 107.3. We propose beginning treatment with a dose of 10 μg/kg subcutaneously, once per day for three consecutive days. Thereafter doses are given as needed to titrate the ANC to around 1000/μL. We did not include criteria for administering rGM-CSF, as we found insufficient evidence for its use in the NICU. If one follows this schema (Fig. 107.2) it will result in little use of rG-CSF in the NICU. Any rG-CSF usage in Neonatology should be focused on patients with the most to gain and least to lose by its application. As additional pertinent investigative work is published, these guidelines shall be modified accordingly.

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Fundamentals of Feto-Neonatal Immunology and Its Clinical Relevance

Akhil Maheshwari and Edmund F. La Gamma

108.1 Introduction

The transition from fetal to neonatal life at birth forms an important functional watershed in the developing immune system. In utero, the fetus is exposed to a steady stream of foreign antigens that are derived mainly from the mother, and must down-regulate its immune response to survive. After birth, however, the neonatal immune system is exposed to a new, more diverse set of antigens and must evolve dichotomous responses to contain the micro-organisms on various cutaneous and mucosal surfaces, and at the same time, develop tolerance to other commensal microbes and dietary macromolecules. During this remarkable transition, while some components of the immune system perform at par with adults, immaturity of the other arms results in a developmentally-regulated state of immunodeficiency. This chapter highlights major quantitative and qualitative differences in the innate and adaptive arms of the neonatal and adult immune systems and provides a brief review of the developing mucosal immune system.

108.2 Clinical Relevance

Preterm neonates have a high susceptibility to bacterial infections, especially of the Gram positive and Staphylococcal variety suggesting a preponderance of vulnerability due to neutrophil function. They are also remarkably deficient in circulating antibody after their trans-placental passage, yet preterm neonates can generate an amnesic response to produce antibodies. Nevertheless, this makes them vulnerable to impaired opsonization (a bacterial vulnerability) and viral dissemination (an uncommon but real biological threat). How these vulnerabilities arise and what opportunities remain to augment defenses can be understood best by an in depth grasp of how the mature capacities of function change over time. Our goal is to highlight those features.

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108.3 Innate Immune System

This section will review the developmental issues relevant to the collection of defense mechanisms the human neonate is born with prior to exposure to environmental antigens and acquisition of adaptive immunity. Cell lines, their maturation and functional capacities or limitations will be defined.

108.3.1 Neutrophils

108.3.1.1 Development

The two major hematopoietic progenitors committed to the neutrophil lineage are the colony-forming units (CFU)-mix, which give rise to a mixture of various leukocyte populations, and the CFU-GEMM, which produce granulocytes, erythrocytes, megakaryocytes, and macrophages. The mechanisms underlying this process of lineage commitment are now beginning to be understood [1]. The effect of biomechanical forces as critical regulators of hematopoiesis is a new field of investigation [2], as is the prevention of apoptosis as a mechanism for hematopoietic progenitor cell growth and development [3]. In the bone marrow, the neutrophil cell lineage includes the early precursors with a capacity for 4–5 five cell divisions, and the later, post-mitotic stages that are in the process of differentiation (Fig. 108.1). In adults, the neutrophil proliferating pool (NPP) contains about 2×10^9 cells/kg body weight and the neutrophil storage pool (NSP) contains about 6×10^9 cells/kg body weight [4]. The NPP and NSP together contain nearly 90% of all neutrophils in the

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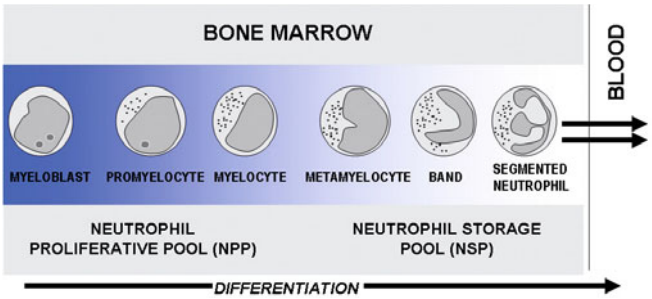

Fig. 108.1 Neutrophil differentiation process in bone marrow

body. The remaining 10% (0.6×10^9/kg) are equally distributed free in the circulation or attached to the microvascular endothelium.

Although umbilical cord blood and fetal blood have a 10–50 fold higher concentration of CFU-GM than does the blood of adults [5], the fetus has an overall much smaller pool of neutrophil progenitors [6]. In the mid-gestation fetus and preterm infant, the NSP is very small in size and can be readily exhausted during sepsis [4]. Studies in experimental animals show that the NSP is considerably smaller in animals delivered prematurely ($1.0–1.3\times10^9$ cells/kg) than at full-term ($1.3–2.5\times10^9$ cells/kg) and in adulthood ($4.5–7.5\times10^9$/kg). The NPP is also smaller, about one-tenth the size (per kg body weight) of adults. Indirect evidence for a small granulocytopoietic pool in the human fetus come from studies of soluble CD16, which is derived from apoptotic neutrophils, and its low concentrations in plasma of preterm neonates may reflect a low total body neutrophil mass. The low sCD16 levels of premature infants reach adult values only by the fourth postnatal week [7].

During acute inflammation, neutrophils are released from the NSP first, and once these stores are exhausted, progressively immature cells are mobilized (known as the left shift of sepsis). Circulating neutrophils persist in the bloodstream for 6–8 hrs and then for a few hrs to several days in tissues. In the fetus, as in the adult, mature neutrophils are stored within the bone marrow and also in the liver and spleen. These maturational relationships and short-half life frequently result in the presenting clinical sign of neutropenia rather than neutrophilia in newborns with bacterial sepsis.

108.3.1.2 Function

Transendothelial Migration

Circulating neutrophils leave the intravascular compartment to enter the tissues in three major steps: margination and rolling on vascular endothelium, attachment to the endothelial cells, and transendothelial migration [8]. Leukocyte traffic is preferentially directed to inflamed areas through regional changes in vascular flow and along concentration gradients of humoral chemoattractants such as chemokines, bacterial products (such as formyl-met-leu-phe or f-MLP), complement fragments (C5a), and leukotrienes (LTB_4). Among chemokines, members of the CXC subfamily with a glutamate-leucine-arginine (ELR) tripeptide sequence (such as interleukin-8 (IL-8/CXCL8)) have a neutrophil-specific chemotactic activity [9, 10].

In inflamed tissues, transient interruptions in the laminar flow of marginating neutrophils cause these cells to tumble and roll on the vascular endothelial surface. This process is mediated through a process of repetitive binding and release of selectins (L-selectin on neutrophils, E- and P-selectin on endothelium) from their sialomucin receptors called addressins. The term selectin is derived from a key lectin domain that interacts selectively with oligosaccharide receptors bearing sialylated carbohydrate moieties. L-selectin is constitutively expressed on neutrophils and is shed after cellular activation [11]. Rolling neutrophils slow down and attach to endothelium through the binding of β_2-integrins to cognate receptors on endothelial cells [12]. The β_2-integrins expressed on neutrophils include the leukocyte function-associated antigen-1 (LFA-1, $\alpha_L\beta_2$, CD11a/CD18), Mac-1 ($\alpha_M\beta_2$, CD11b/CD18), and the p150, 95 ($\alpha_X\beta_2$, CD11c/CD18), which bind endothelial receptors such as the intercellular adhesion molecule-1 (ICAM-1) and ICAM-2. LFA-1 binds to both ICAM-1 and ICAM-2, whereas Mac-1 and p150, 95 bind exclusively to ICAM-1. These neutrophils undergo activation on the endothelial surface and migrate through the capillary/venular wall, a process that involves the platelet-endothelial cell adhesion molecule (PECAM1, CD31), the integrin-associated protein (CD47), and a series of other junctional molecules [13].

Compared to neutrophils from adults, neutrophils from both term and preterm infants adhere poorly to the endothelium. Neonatal neutrophils have lower selectin expression than adults at birth [14], which might be further reduced by perinatal stress such as in birth asphyxia [15]. In addition, neonatal neutrophils display defective shedding of L-selectin [14]. This combination of decreased expression and impaired shedding of L-selectin impairs the ability of neutrophils to roll on the endothelial surface, thereby limiting the recruitment of circulating neutrophils into the tissues. Neutrophil-endothelial adherence and neutrophil transmigration is further limited in neonates due to a developmental deficiency of Mac-1 (CD18/CD11b), one of the β_2 integrins [16]. The transendothelial migration of neutrophils is also limited, at least in immature cells released from the NSP during sepsis, by reduced deformability of the neutrophil membrane and cytoplasm [17]. For these reasons, it is generally believed that the propensity of neonates to bacteremia is more typical than a proper localization and abscess formation.

Chemotaxis

Once outside the blood vessel, neutrophils migrate along concentration gradients of various chemoattractants such as IL-8 (and other ELR^+ CXC chemokines), f-MLP, and C5a [9].

These chemotactic stimuli bind to high-affinity G-protein-coupled receptors on the leukocyte surface, and minute spatial gradients in chemoattractant concentrations can cause the receptors to be distributed asymmetrically towards the migrating neutrophil pseudopodium. Cellular movement involves a number of intracellular signaling pathways and cytoskeletal proteins. A chemoattractant hierarchy has been reported wherein bacterial products are preferred over host chemokines [18].

Neutrophils from both term and preterm neonates have an impaired chemotactic response, migrating at only about half the speed traveled by adult cells [19, 20]. While neutrophils from term infants achieve normal chemotactic function by 2 wks after birth, such postnatal neutrophil maturation begins 2–3 weeks after birth in immature preterm infants and proceeds very slowly [21]. Neutrophils from preterm infants born at 34–36 wks gestation achieve normal chemotaxis by 40–42 weeks of post-conceptional age. In more immature preterm infants (<34 wks), neutrophil chemotaxis improves with time, but remains impaired in comparison to adults even at 42 wks PCA [22]. Although minor infections may enhance chemotaxis in neonates, the migratory responses of neonatal neutrophils may become further depressed during systemic Gram-negative sepsis [23].

Neonatal neutrophils bind various chemoattractants normally. However, chemoattractant-induced membrane depolarization, calcium transport, and sugar uptake are relatively less efficient. The chemotactic defect in neonatal neutrophils may be multi-factorial, affected by factors such as a larger, poorly motile neutrophil subpopulation, impaired calcium mobilization, and aberrations in intracellular signaling pathways such as NF-κB activation [24, 25]. Lower Mac-1 expression can also impede chemotaxis due to impaired neutrophil interaction with the extracellular matrix [24]. Inability to effectively direct neutrophils to the bacterial source contributes to the neonatal vulnerability to septicemia.

Phagocytosis

Phagocytosis is a specialized form of endocytosis directed at engulfing solid particles into an internal phagosome. This internalized phagosome "matures" through interactions with the endosomal compartment and eventually fuses with a lysosome for killing of internalized microorganisms and terminal degradation of the cargo [26]. Phagocytosis is more efficient when the target is opsonized by specific immunoglobulin G (IgG) or complement factors, which may act by neutralizing inhibitors of phagocytosis such as the capsular polysaccharide or by rendering the microbial surface more hydrophobic. Neutrophils express receptors for IgG ($F_c\gamma$ receptors I-III, or CD16, CD32, CD64), C3b (CR1), and iC3b (CR3). In some instances, microorganisms may be ingested without opsonization through lectin-carbohydrate (lectins on bacterial fimbriae interact with neutrophil glycoproteins), protein-protein (proteins such as filamentous hemagglutinin that express the arg-gly-asp or the RGD amino acid sequence bind to integrins), and hydrophobic-protein (bacterial glycolipids and neutrophil integrins) interactions [26].

The interaction of IgG or complement receptors on the neutrophil surface with the opsonized particle trigger cytoskeletal rearrangements to enclose the opsonized particle within a phagosome. Phagocytosis is most efficient when organisms are coated with both IgG and C_3, which allows cooperative interaction of cognate receptors for both the opsonins. As mentioned above, neutrophils express integrin receptors for matrix proteins with the RGD tripeptide motif (such as fibronectin, laminin, and collagen), and ingest C_3-coated particles more efficiently when adherent to surfaces coated with these RGD-bearing proteins [26, 27].

Preterm neutrophils have impaired phagocytosis, which corrects only in the late third trimester to become comparable to adults [19]. Preterm neutrophils ingest particles more slowly and ingest fewer bacteria (such as *E. coli*). The lack of opsonic activity is an important consideration, as preterm infants often have lower concentration of specific antibodies [28]. Compared to term neonates and adults, preterm neutrophils also have decreased expression of CD16 ($F_c\gamma$RIII) and CD32 ($F_c\gamma$RII), the two most abundant neutrophil IgG receptors [29]. Whereas CD16 expression normally increases to adult levels over the first three weeks of life, CD32 deficiency may or may not correct with time [30].

Intracellular Killing

The phagolysosome provides an enclosed space in which an ingested microbe is exposed to high concentrations of toxic substances, while limiting the exposure of the phagocyte and other cells to these potentially injurious agents [26]. The major killing mechanism in neutrophils involves the generation of reactive oxygen species (ROS) in a respiratory burst. An NADPH-dependent oxidase localized on the cell membrane (and therefore, the phagosome membrane) reduces molecular oxygen (O_2) to a superoxide anion ($O^{\bullet}_2^-$) [31]; subsequent generation of peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$, formed in the presence of iron) also contributes to the microbicidal capacity of neutrophils [32]. These oxygen-dependent bactericidal mechanisms can be broadly divided into myeloperoxidase (MPO)-independent (such as hydrogen peroxide) and MPO-dependent (MPO catalyzes reactions between H_2O_2 and halides to form highly reactive products) [33]. H_2O_2 is a weak bactericidal agent per se, but the MPO-H_2O_2-halide system increases its efficacy by nearly 50-fold. The bactericidal effects of free oxygen radicals are due to oxidizing effects on various components of the bacterial cell wall [32].

Neutrophils also have elaborate non-oxidative killing mechanisms such as low pH (as low as 6.0), defensins, bactericidal/permeability-increasing protein (BPI), lactoferrin, lysozyme, and a variety of cationic proteins. Defensins are

broad-spectrum antimicrobial peptides with activity against gram-positive and gram-negative bacteria, fungi, and enveloped viruses and are also released in the gut by Paneth cells [34]. BPI binds lipopolysaccharide (LPS) and blocks its effects, can damage the outer membrane of gram-negative bacteria, and has some opsonic activity. Lactoferrin, an iron chelator, is bacteriostatic as it deprives bacteria of the iron required for growth. Lactoferrin is also involved in neutrophil degranulation, in oxygen radical production, and in granulocytopoiesis. Lysozyme hydrolyzes a glycoside bond in the bacterial cell wall peptidoglycan. Primary granules also contain other cationic antibacterial proteins such as azuricidin, indolicin and cathelicidins [26, 35].

The respiratory burst is depressed in preterm neutrophils, which explains the observed developmental defects in intracellular killing of bacterial pathogens such as *Staphylococcus aureus* or *E. coli* [36]. The killing capacity cannot be fully explained on the basis of low opsonic activity in preterm plasma and improves only as a function of gestational age [19, 37, 38]. The neutrophil respiratory burst in infants born at 24–28 wks is clearly less robust than in those born at 29–35 wks and takes about 2 months to correct. However, neutrophils from preterm infants continue to have an overall weaker oxidative burst than adults and may not show any improvement in critically-ill preterm infants [39]. Neonatal neutrophils are also less effective in killing group B streptococci, although the data on candidacidal activity are conflicting. The antiviral activity of neonatal neutrophils is also diminished compared with that of adults. Indeed, the most commonly recovered bacteria in extremely low birth weight/extremely low gestational age neonates (ELBW/ELGAN) is a commensal organism *Staphylococcus epidermidis*.

Degranulation

Neutrophils contain two major types of granules (Fig. 108.2): (1) the azurophilic granules (stain positive with the azure A dye) and (2) specific granules (do not stain with azure A). Azurophilic granules contain myeloperoxidase (MPO), proteolytic enzymes such as cathepsins, proteinase-3, and elastase, and antimicrobial proteins such as defensins and the bactericidal permeability-increasing protein (BPI). These granules release their contents into the phagolysosomes and are involved in intracellular killing. The specific granules contain antibacterial agents such as lactoferrin and lysozyme, receptors for complement components, and bacterial products such as f-MLP. Specific granules fuse with the cell membrane to release their contents by exocytosis, and also bring functionally important membrane proteins such as integrins, cytochrome-b_{558}, and receptors for chemotactic agents and opsonins to the cell surface. Specific granules play an important role in extracellular killing [40].

Neutrophils from term neonates have granule contents and degranulation responses similar to adults [41]. However, neutrophils from preterm infants have a lower capacity to release BPI, elastase, and lactoferrin than in adults and term infants [19, 42]. Collectively, neutrophil line immaturities and limited function accounts for a substantial component of neonatal susceptibility to invasive bacterial infections.

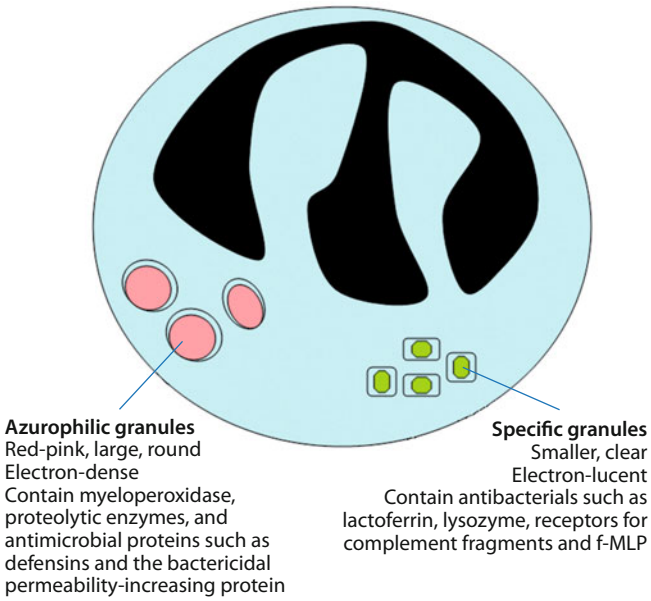

Fig. 108.2 Types of granules in neutrophils

108.3.2 Monocytes and Macrophages

108.3.2.1 Development

Embryonic macrophages are first seen in the yolk sac at 3–4 weeks of gestation. Unlike macrophages in the fetus and the adult, which are derived from circulating monocytes, these large-sized histiocytic cells develop in the early embryonic period from yolk sac progenitors prior to the first appearance of monocytes [43, 44]. At 5 weeks of gestation, two distinct cell lineages with a dendritic/macrophage structure can be identified in the yolk sac, mesenchyme and the fetal liver. The larger subgroup is MHC II-negative, and there is only a minor population that expresses these antigens. MHC II-negative cells also appear in the thymic cortex, in the marginal zones of lymph nodes, in the splenic red pulp, and in the bone marrow [45]. A few MHC II-positive cells are seen in the liver at 7–8 weeks of gestation, the lymph nodes at 11–13 weeks of gestation, and the T-cell areas of the developing thymic medulla by 16 weeks of gestation. Subsequently, the numbers increase gradually, and MHC class II-positive cells are also seen in the skin and gastrointestinal tract [45].

During the second month of gestation, as hematopoiesis becomes established in the fetal liver, monocytes are seen in

high proportions and constitute nearly 70 percent of all hematopoietic cells [46]. Over the next 6 weeks, as the erythroid cells predominate, this proportion falls to 1–2 percent [46]. The first monocytes in circulation are not seen until about the fifth month of gestation [46], and remain uncommon until the bone marrow becomes the predominant site of hematopoiesis [47]. At 30 weeks, monocytes comprise 3–7 percent of hematopoietic cells [48]. Term cord blood studies show a relative monocytosis, which persists during the neonatal period. The absolute monocyte counts tend to decline gradually from 1340–2200/μL in the first week to about 700 in the third week [49]. We are currently analyzing data from a large cohort of neonates with more than 100,000 blood counts to define the normal ranges of absolute monocyte counts.

Information on tissue macrophage kinetics in the neonatal period is mainly from autopsy studies. The size of the macrophage pool varies in different organ systems. In the gastrointestinal tract, macrophages appear in the lamina propria as early as 10 weeks of gestation and a sizable macrophage population can be seen during midgestation [44]. In contrast, the alveolar macrophage population remains small in the fetus and expands rapidly during the early neonatal period [50]. This increase may result both from an influx of monocytes from the circulation as well as from clonal expansion in situ.

Monocytes and macrophages can be identified using immunocytochemistry for the macrophage marker HAM56, cellular constituents such as nonspecific esterase and peroxidase, innate immune receptors such as CD11b, and the MHC complex [51–54]. A lower proportion of cord blood macrophages stain for esterase, although there are no discernible differences in peroxidase activity [52]. Neonatal monocytes have lower expression levels of the aforementioned surface markers, except for CD14 [53]. In certain tissues, such as in the gastrointestinal tract, resident macrophages are characteristically downregulated for the expression of innate immune receptors such as CD14 [55].

Due to the extraordinary importance in this particular lineage in epithelial surface barrier protection, the propensity of ELBW/ELGAN patients to pneumonias, necrotizing enterocolitis and invasive skin infections merits consideration.

108.3.2.2 Function

Transendothelial Migration, Chemotaxis, Phagocytosis, and Respiratory Burst

Unlike neutrophils, the major host defense functions of monocytes in cord blood of term infants are largely intact. Cord blood monocytes show adherence, random migration, chemotaxis, bactericidal activity, phagocytosis-associated chemiluminescence, production of superoxide anion (O-2) and generation of hydrogen peroxide at levels comparable to those of cells from healthy adult volunteers [56, 57]. The ability of fetal and neonatal monocytes to kill a variety of pathogens including *S. aureus*, *S. epidermidis*, *E. coli*, and *C. albicans* appears to be equivalent to that of adults [56, 58].

Resident macrophages are often the first phagocytic cells of the innate immune system to encounter invading pathogens breaching the various epithelial surfaces of the skin, gut and lung. These cells serve important host defense functions through phagocytosis, and also as sentinel cells that regulate local inflammatory responses by producing various cytokines and chemokines [59, 60]. Most studies on monocyte/macrophage cell function have been done on cord blood, and fetal cells have not been studied to the same extent so far. Term cord-blood monocytes produce IL-1, IFN-α and TNF-α in concentrations that are comparable to adults, but the levels of IFN-γ, IL-8, IL-10, and GM-CSF are lower. These cells also produce lower concentrations of extracellular proteins like fibronectin, and bioreactive lipids like leukotriene B_4 [61, 62]. Impaired monocyte secretory functions in neonates may be partially responsible for poorer cytokine responses of neonatal T-cells.

Emerging evidence indicates that macrophages are dynamic and heterogeneous cells, which are polarized into the classically-activated M1 macrophages that express various inflammatory signals, and the more-recently described M2 macrophages that function with an anti-inflammatory profile [63]. Although the effect of development on macrophage polarization is not known, the relative inability of cord blood monocytes (versus monocytes from adults) to mount a robust inflammatory cytokine response is intriguing [64].

108.4 Adaptive Immune System

108.4.1 Dendritic Cells

108.4.1.1 Development

Dendritic cells (DCs) are a discrete leukocyte population with a highly developed antigen-presenting function. DC populations have been grown from separated hematopoietic precursors, suggesting that there is a common granulocyte-monocyte-dendritic cell progenitor [65]. Cells with a dendritic/macrophage structure are present in the yolk sac, mesenchyme and the liver at 4–6 weeks of age. DCs are detectable in skin by 6–7 weeks of gestation.

DCs initially derived their name from their distinctive morphology, with numerous fine dendritic cytoplasmic processes commonly found penetrating epithelial bound organ surfaces. However, phenotype alone is not sufficient to define these cells in view of functional differences between the subpopulations. A working definition requires DCs to be able to stimulate T-cells, home to T-cell dependent lymph node areas, be able to pinocytose and have characteristic cell surface antigens.

Human peripheral blood DCs mainly include two subgroups [66]: (1) myeloid DCs (or mDCs) are $CD11c^+$ cells that express myeloid markers such as CD13, CD33, and CD11b; and (2) plasmacytoid DCs (or pDCs) are $CD11c^-$ and have a plasmacytoid morphology with well-developed rough endoplasmic reticulum and Golgi apparatus.

108.4.1.2 Function

Cord blood-DCs represent about 0.3% of all mononuclear cells. Most studies show an increased number and proportion of pDCs in cord blood compared to adult peripheral blood, with pDC:mDC ratios of 1–3:1 that contrast with the usual 1:2 ratio in adults [67].

Due to the low frequency of DCs in peripheral blood, most studies of neonatal DCs have been carried out using *in vitro* monocyte-derived dendritic cells (MDDCs). Compared to pDCs from adults, cord blood DCs exhibit low or no basal expression of co-stimulatory molecules CD40, CD80 or CD86, show an impaired maturational response following stimulation with agonists for various toll-like receptors (measured as an increase in the expression of co-stimulatory molecules and production of IFN-alpha, TNF-alpha, IL-1, IL-6 and IL-12), and perform poorly at accessory function [68]. Cord-blood dendritic cells have lower expression levels of ICAM-1 and MHC antigens than in adults. These cells are also poor stimulators of mixed lymphocyte reactions, regardless of whether cord or adult mononuclear or T-cells were used as the responders. How precisely these cells contribute to the susceptibility of neonates to maturation of adaptive immunity, prevention of invasive illness and barrier dysfunction is an area in need of further exploration.

108.4.2 T-Lymphocytes

108.4.2.1 Development

The thymus arises at about six weeks of gestation from the third branchial arch, with the cortex arising from its ectodermal layer and the medulla from the endoderm. Lymphoid cells migrate over the next 2–3 weeks, initially from the yolk sac and fetal liver, and then from the bone marrow to colonize the fetal thymus [69]. These prothymocytes interact with the stroma, proliferate actively, and are triggered to differentiate with expression of the first T-cell-specific surface molecules (e.g., CD2, and later CD4 and CD8) [70, 71]. A clear delineation of the thymic cortical and medullary regions occurs at 12 weeks of gestation; Hassall's corpuscles appear shortly thereafter [72, 73]. The most immature thymocytes are found in the subcapsular cortical region, and cells move into the deeper layers as they mature [72].

The early prothymocytes do not express CD3, the T-cell receptor (TCR), CD4, or CD8 and are often referred to as triple-negative thymocytes [74]. The progeny continue to divide and rearrange their TCR genes, and since these cells express both CD4 and CD8, they are now called double-positive [72, 74]. They undergo positive selection by self-major histocompatability complex (MHC) restriction, and more than 95 percent (about 50 million) cells die each day during this stage [74]. Negative selection occurs next, and is mediated by the bone marrow-derived antigen-presenting cells (APC) (e.g., dendritic cells and macrophages), which eliminate autoreactive cells either by clonal deletion or clonal anergy [75]. As these thymocytes mature and reach the medulla, they express only one of the CD4 or CD8 antigens. These single-positive T-cells migrate from the thymus to the peripheral lymphoid organs at about 14 weeks of gestation [72]. By 15 weeks, human thymocytes express a complete set of TCRs [72, 76].

During fetal life, thymus is the largest lymphoid tissue in terms of body proportions. It is about two thirds its mature weight at birth, and reaches its peak mass at around 10 years of age. Subsequently, it continues to involute and is replaced by adipose tissue [77].

108.4.2.2 T-Cell Receptor (TCR) Repertoire

The TCR is composed of two distinct functional subunits, each specialized for a different function [78]. The first, highly polymorphic, is uniquely structured for each T-cell for antigen recognition; it is composed of two polypeptide chains, α and β (except in a specific T-cell subset where it consists of γ and δ chains) [78]. The second, also known as CD3, is a trimolecular complex, involved in signal transduction and cellular activation. The extracellular region of the TCR resembles an immunoglobulin (Ig) Fab fragment, and derives its structural diversity from recombinatorial permutations involving a set each of Variable (V), Diversity (D), and Joining (J) gene segments [79, 80]. The variable domain is situated in the N-terminal end of the α/β (or γ/δ) chains, whereas the C terminal is the constant region [78]. The variable domains consist of V, D, and J elements in the β chain, and V and J in the α chain [81]. The antigen-binding sites are formed by three complementarity-determining regions (CDRs). CDR3, the most extensive of these segments, serves as a key site for antigen recognition [82].

By midgestation, all the TCR Vβ families (V here refers to the variable domain of the β chain, and should be recognized as different from the V gene segments mentioned above) are expressed, but they have shorter CDR3 regions. This is primarily due to limited expression of the enzyme terminal deoxynucleotidyl transferase (Tdt), which induces N-terminal diversity and, consequently, CDR3 heterogeneity. In term infants, the T-cell Vβ repertoire is similar to that seen in adults [82, 83]. Cord blood T-cells from term infants are also able to expand the TCR β repertoire on stimulation with bacterial toxins [83]. It is the central position of T-cell function in the maturing immune system that places the capacity to

adapt to a changing environment central to successful maturation of immune defenses. This is primarily a time and antigen experience-dependent process influenced by the maternal inoculation with flora during a normal birth as well as the subsequent clinical and home environments as influenced by clinicians and their use of antibiotics or other teleologically relevant experiences.

108.4.2.3 Circulating T-Cells

T-cell subpopulations gradually increase in number beyond 19 weeks' gestation, and continue to rise after birth to peak at about 6–9 months. The numbers subsequently decline, and adult levels are finally reached at 6–7 years of age [84]. In term neonates, CD4+ cells constitute a higher proportion of T-cells than adults. CD8+ cells, on the other hand, are fewer both in terms of their absolute number and as a percentage of total T-cells. The CD4/CD8 ratio, consequently, is as high as 4.9:1 during the perinatal period, and declines to adult values of approximately 2:1 only by 4 years of age [84]. Preterms have significantly higher numbers of CD4+ T-cells, but the number of CD8+ T-cells does not seem to change with gestational age [85]. These numbers decrease with perinatal distress, but normalize by 3 weeks of age except in some very-low-birth-weight infants [85].

Peripheral T-cells in the fetus and neonate may be in a relatively immature transitional state; in cord blood, nearly 85% of T-cells express CD38 (compared with less than 5% of adult cells), but lack other markers of activation. Cord blood T-cells also differ in being predominantly naïve (80–90% express the CD45RA phenotype, compared with only 40–60% of the adult cells) [86]. The percentage of memory T-cells (CD45RO) increases in healthy infants during the first few years of life, but reaches adult levels only by the second decade [86]. Neonatal T-cells also have lower expression of CDw29 and CD11b, providing further evidence to a lack of previous stimulation [86]. The ratios and relationships of T-cell subtypes and the clinical relevance to invasive disease is an area of perinatal immunology that is in need of further clarification.

108.4.2.4 Function

Proliferation

Cord blood T-cells from premature infants have a limited capacity for mitogen-induced proliferation but these defects are corrected by full-term [86, 87]. Similarly, proliferative responses to monoclonal antibodies against T-cell markers (e.g., CD3, CD2) are dramatically less than adult lymphocytes, and resemble adult naïve T-cells [88]. These responses improve as the numbers of peripheral blood memory T-cells increase. When tested with allogeneic cells (mixed lymphocyte reaction), however, cord blood lymphocytes respond better, though still somewhat less than cells from adult subjects [86, 87, 89, 90].

Cytokine Production

Neonatal concentrations of pro-inflammatory cytokines like IL-1, IL-6, TNF-α, IFN-α, and IFN-β are comparable to adults, and also increase similarly during sepsis [91, 92]. Premature infants, however, are known to produce lower amounts of TNF-α and IFN-α compared to those born at term [93, 94]. Among the cytokines involved in adaptive immunity, only IL-2 concentrations are comparable; others like IL-4, IL-5, IL-10, IL-15 and IFN-γ are known to be significantly lower than adults [93–96]. Transforming growth factor (TGF)-β_1 and macrophage inflammatory protein-1alpha (MIP)-1α, both of which play a negative role in hematopoiesis, are also present in much lower concentrations [97].

The concentrations of hematopoietic colony stimulating factors including IL-3, Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), Granulocyte-CSF and Monocyte-CSF are slightly lower to comparable to adults, but even full-term infants are known to be unable to mount adequate responses during stress [98]. Chemokines, which have emerged as important regulators of leukocyte trafficking, are currently an important area of study. The concentrations of some of these are known to be comparable to adults, including IL-8/CXCL8, epithelial neutrophil attractant (ENA)-78/CXCL5, growth-related oncoprotein (GRO)-α/CXCL1, eotaxin/CCL11, and RANTES (regulated upon activation, normal T-cell expressed and secreted)/CCL5 [99].

Most of these deficiencies are caused by altered regulation of post-transcriptional mRNA processing [97]. It is believed that lower concentrations of some of these cytokines are caused by a relative paucity of memory or primed T-cells. However, genetic and other individual traits such as atopy are also likely to be important determinants of the cytokine response [100].

During development, naïve T-cells differentiate into distinct effector T-helper (Th) subsets, a process in which cytokines play a critical role. These differentiated T-cells were originally categorically designated Th1 and Th2 cells based on distinct functional properties and the cytokines that drive their development [101]. Th1 type cytokines, such as IFN-γ and IL-2 play a key role in initiating early resistance to pathogens, and induction of cell-mediated immunity. Th2 cytokines drive the system toward immune tolerance rather than toward defense from microbial infections. Accumulating evidence suggests that Th1 responses in newborns are compromised at several steps including deficient production of Th1 type cytokines by neonatal CD4+ T-cells and hyporesponsiveness of neonatal macrophages to stimulation by IFN-γ. These deficiencies contribute to the apparently weak cellular immunity in newborns biased towards a Th2 type response [102].

Since the original Th1 and Th2 description, a differentiated T-cell population, Th17 cells, has been shown to have a pathogenic role in allergic, autoimmune and other chronic inflammatory diseases [103, 104]. Th17 cells have also been shown to play a protective role in immunity to infection, where they take on Th1-like effector functions to promote pathogen clearance by enhancing neutrophil recruitment to sites of infection and activating macrophages [103–105]. While the role of Th17 cells early in life remains unclear, cord blood mononuclear cells have a limited capacity to produce IL-17 [106]. In very premature infants with a history of serious bloodstream infections (BSI), we found lower serum IL-17 levels than their counterparts who never developed BSI.

The fetus occupies a unique immunologic position where the Th2 phenotype predominates in utero and transitions to a more Th1 functionality following birth. It is generally considered to be a core process of evolutionary significance where both mother and fetus maintain a high level of immunologic suppression to enable continuation of pregnancy.

Antigen-Specific Responses

The response of T-cells to specific antigens can also be assessed in terms of proliferation or cytokine production. These responses usually require previous exposure to the corresponding antigen, and are generally not detected at birth or from cord blood unless there was an intrauterine exposure. Neonatal T-cells do, however, respond well to certain antigens such as tetanus/diphtheria toxoids, influenza, and mycobacterial antigens [87]. In response to superantigens, cord blood T-cells produce lesser amounts of IL-2 [107]. However, after stimulation, the percentage of $V\beta_2$+ T-cells (which determine potential reactivity to superantigens) and the number of memory T-cells increase significantly just like adults [107]. But unlike adult T-cells, cord blood T-cells are unable to respond if restimulated with the superantigen. This tolerance induction in cord blood T-cells may again be due to the underlying immunologic naiveté [107, 108].

108.4.2.5 Other Subgroups

Cytotoxic T-Lymphocytes (CTLs)

CTLs are important in host defense against intracellular infections, in allograft rejection and tumor cell surveillance [109]. CTLs utilize two well-established mechanisms for cell lysis, one involving release of extracellular mediators (such as the pore-forming perforin/granzyme system), and a second fas/fas ligand dependent pathway that leads to target cell apoptosis [110].

CTL cytotoxicity is evident by 18 weeks of gestation, but is far less efficient that adult cells even at term (<20 percent of adult CTL activity) [111]. Perforin expression in neonatal CTLs is about 30 percent of adult levels. CD28, which is a T-cell activation marker, is also expressed to significantly lower levels [90]. Similar results are also observed in other assays; neonatal cells showed only 33 percent of lectin/mitogen-dependent cytotoxicity of adult cells [112]. Circulating inhibitors such as a-fetoprotein and prostaglandins may also lead to lower CTL activity in neonates [112].

γδT-Cells

The γδ T-cells represent a distinct functional subset, with a majority lacking surface expression of both CD4 and CD8 [113]. These cells are detectable in the fetal thymus and liver at 6–8 weeks of gestation, and comprise nearly 10% of the peripheral blood T-cells at 16 weeks [114]. Subsequently, the numbers decline gradually to reach about 3% at term [115]. These cells are present mainly on skin and mucosal surfaces [113]. Although the exact functions of these T-cells are not well understood, they can lyse target cells with the perforin/granzyme system like the cytotoxic T-cells, and can secrete cytokines like interferon (IFN)-γ and tumor necrosis factor (TNF)-α upon activation. The cytotoxicity of neonatal γδ T-cells is significantly less than adults [116].

Fetal γδ T-cells have a more diverse repertoire but a more limited junctional diversity than adults. This diversity is retained throughout the first year of life, and then decreases gradually over the first decade of life [116]. Overall, however, γδ T-cells have a relatively restricted repertoire in comparison to the αβ T- or B-cells [117].

T-Regulatory Cells

T-regulatory cells (Tregs) downregulate T-cell responses to both foreign and self antigens, thereby playing an important role in balancing Th1/Th2 effector lineages [118]. Treg cells, including both natural $CD4^+$ $CD25^+$ Tregs as well as the IL-10-producing Tregs, express the forkhead/winged-helix family transcriptional repressor-p3 (Foxp3) [119], a commonly-used but not entirely specific marker for Tregs [120]. Although Tregs have been detected in cord blood, current knowledge about Treg function early in life is limited and thus their relevance to immune defense of the newborn is uncertain.

108.4.3 B-Lymphocytes

108.4.3.1 Development

B-cell progenitors, pro-B-cells, are derived from pluripotent hematopoietic cells in the bone marrow [121]. The first

recognizable B-cell progenitor, the large pre-B-cell, is characterized by the presence of cytoplasmic μ heavy chains [121]. Immature B-cells undergo a selection process analogous to T-cells to eliminate self-identifying clones (clonal selection, clonal deletion), although there may also be other mechanisms to maintain self-tolerance [122]. Once B-cells begin to express surface IgM (sIgM), they are ready to leave the bone marrow to enter the peripheral circulation [123].

Pre-B-cells can be identified in the fetal liver as early as 7 weeks of gestation, and in the marrow by 12 weeks. sIgM+ B-cells are found in the fetal liver by 9 weeks and in the bone marrow, peripheral blood, and spleen by 12 weeks. B-cells with sIgA, sIgG, and sIgD isotypes appear between 10 and 12 weeks. There is also increased traffic to the lymphoid tissues, and by 22 weeks, the proportion of B-cells in the spleen, peripheral blood, and bone marrow is similar to that in adults. By 30 weeks, there are no detectable pre-B-cells in the fetal liver, and bone marrow becomes the exclusive site for B-cell maturation. Plasma cells are not generally found until 20 weeks' gestation. IgM/IgD+ B-cells populate the lymph nodes by 16–17 weeks' gestation and the spleen by 16–21 weeks. In fetal lymph nodes, primary nodules develop around the follicular dendritic cells by 17 weeks' gestation [124]. Considering the extreme degree of premature birth and survival in this era even below 24 weeks, it is perhaps no surprise that effective production of antibody for opsonization and enhanced cytotoxicity is limited.

108.4.3.2 Immunoglobulin Repertoire

Receptor diversity in the antigen binding site originates from DNA recombination involving various V, D, or J gene segments giving rise to a large number of V(D)J permutations [80]. Additional receptor diversity is generated by imprecise gene segment joins, additional nucleotides added to the splice junction of the VDJ joins by the enzyme terminal deoxynucleotidyl transferase (TdT), and somatic mutations (for B-cells only, not T-cells). Thereafter, these VDJ or VJ (light chains) units join to their respective constant region gene segments [125].

In the fetus and neonate, the Ig repertoire is relatively restricted. During early and mid-gestation, certain heavy chain V gene segments are preferentially expressed. Early in fetal life, the most J_H proximal V_H gene segments are preferentially utilized, and consequently, the CDR3 region of the rearranged VDJ gene segment is shorter than that in adults. This leads to relatively limited junctional diversity, but this altered architecture of the antigen-binding site may also allow greater polyspecificity of antigen binding (at a cost of lower antibody affinity) [126]. The utilization of V_H gene families spreads out more evenly with increasing gestation. However, even at term, cord blood B-cells have a higher usage of V_H genes of the V_H 1 and V_H 5 family with decreased V_H 3 use compared with adult B-cells [127]. In general, the antibody response in neonates is of low affinity, and restricted to the IgM isotype. The somatic mutation of the heavy and light Ig variable region genes and the selection of higher affinity antibody-producing B-cells is limited at birth but increases very slowly after 10 days of age [128]. It is significant that the maturation of antigen presenting cells and functional limitations of B-cells reduces the capacity of effective defense mechanisms during the period of novel exposure to nutrient derived antigens and gut flora yet eventually acquires the ability to accurately discern self from diet.

108.4.3.3 Circulating B-Cells

At birth, the proportion of B-cells is similar to that of adults, but the absolute number of B-cells is significantly higher [129]. The number peaks at about 3–4 months of age, and then declines to adult levels by 6–7 years of age [130]. Preterm infants have comparable B-cell numbers to the term infants [131]. However, the number is smaller in growth-retarded infants. Unlike adults, most B-cells in cord blood express activation markers (CD25, CD23, transferrin receptor) [132].

108.4.3.4 Function

Immunoglobulin Production

The fetus and the neonate are capable, although at a lower intensity than adults, of mounting antigen-specific antibody responses. The presence of allergen-specific IgEs in cord blood, anti-tetanus IgM in cord sera of newborns whose mothers were vaccinated during pregnancy, and reactivity to *Ascaris* antigens in the event of maternal infestation are some examples [133, 134].

However, this response remains immature [124]. They may be unable to respond to all the antigens in a vaccine, and often have delayed isotype switch [135]. It appears that the interval from birth is a more important determinant of antibody response than the gestational age. Both preterm and term infants immunized with diphtheria toxoid at 0–10 days of age had poorer responses than similarly immunized adults, but the response was better when vaccination was deferred until 1–2 months of age [135]. With certain antigens like hepatitis B, however, premature infants may show a relatively poorer early response than their term counterparts, although these deficiencies correct during later infancy [136]. Collectively, these observations suggest that many of the maturational signals needed to achieve a full capacity of host defenses arise from evolutionary appropriate cues received from the neonatal environment forcing the irrevocable expansion of a wide diversity of immune capacities. Thus, the newborn is effectively, not solely limited by its ontological immaturity of cellular and humoral capacities.

Serum Immunoglobulin Levels

Serum Ig levels remain very low until 18–20 weeks' gestation. Most of the newborn's serum immunoglobulins are derived from active transplacental transfer of maternal IgG (particularly IgG1 and IgG3) during the third trimester [137]. In the full-term neonate, serum IgG levels are equal or even higher than maternal serum IgG levels, but in the preterm, who missed these maternal antibodies, the levels are lower.

Hobbs and Davis found that nearly all infants born before 32 weeks' gestation had serum IgG levels less than 400 mg/dL at birth (compared with term infants who had serum levels around 1000 mg/dL) [138]. The levels fall after birth (through normal catabolism) to a nadir of 300–500 mg/dL between 3 and 5 months of age, when the infant starts producing increasing amounts of his/her own. Because of starting at a lower level, this nadir may be much lower, and earlier, in preterm infants [139]. Cord blood Ig levels also tend to be lower in growth retarded neonates [140].

The serum levels of IgA, IgM and IgE are very low even in term infants, since these do not cross the placenta. However, when faced with an intrauterine infection, the fetus is definitely capable of producing appreciable amounts of IgM [141]. For many years it was believed that augmenting the neonatal repertoire with exogenous pooled IgG could augment immune functions. Unfortunately, data proving efficacy of this approach for prevention or intervention for bacterial infections has not proven compelling; nevertheless, use of high titer Ig for mitigating perinatal transmission of hepatitis B is well established.

Taken together, it is clear that an improved understanding of these IgG relationships may some day be exploited to enhance bacterial opsonization.

108.4.3.5 Other Subgroups

CD5 Expressing B-cells

B-cells with surface expression of CD5, which is a T-cell antigen, may represent a functionally and ontogenically distinct subset. Some believe that CD5 positivity defines a so-called B-1 subset of cells, distinct from the conventional adult B2 population by virtue of their earlier appearance in ontogeny, capacity for bone marrow-independent self-renewal, and constitutively expressing signal transducer and activator of transcription-3 (STAT3) [142]. B-1 cells express the B-cell-lineage antigens CD19 and CD45R, although CD45R is present at lower levels on B-1 cells than on B-2 cells [143–145]. B-1 cells in the peritoneal and pleural cavities can be identified by their unusual CD11b+ sIgMhi sIgDlow phenotype and can be further subdivided on the basis of differential expression of the cell-surface antigen CD5, into CD5+ CD11b+ $sIgM^{hi}$ $sIgD^{low}$ B-1a cells and CD5- CD11b+ $sIgM^{hi}$ $sIgD^{low}$ B-1b cells [146].

These cells are the predominant B-cell type during fetal life, and have a distinctive anatomic localization in the fetal spleen and the peritoneal cavity. They appear in the spleen at 15 weeks, and are seen in the lymph node primary follicles at about 17 weeks of gestation [147]. In adults, CD5 expression can be found on about 25–35% of total B-cells and 1–7% of all the peripheral blood mononuclear cells. In contrast, CD5+ B-cells represent approximately 90% of the total cord blood B-cells. This number decreases to 75–80% during infancy, and reaches adult levels only by late adolescence [147].

The exact function of B-1 cells in the fetus is still unclear. The functional characteristics of B-1 cells such as the unique localization, broad polyspecificities, and a restricted Ig repertoire have been considered to indicate a role of these cells in the innate, rather than in adaptive immunity [148]. Unlike follicular B-2 cells that respond to protein antigens, and with T-cell help, undergo immunoglobulin heavy chain class-switching and affinity maturation, B-1 cells respond mainly to T-cell-independent immunogens that include carbohydrate antigens [143–145].

Recent observations indicate that the two types of B-1 cell, B-1a and B-1b, show functional differences during the immune response. B-1a cells spontaneously secrete IgM, which provides a first line of defense against certain encapsulated bacteria, such as *Streptococcus pneumoniae*, whereas antibody production by B-1b cells is induced and has a role in the ultimate clearance of the pathogen and in providing long-term protection [146, 149, 150].

108.4.4 T- and B-Cell Interaction

T-cell signals are crucial for the proliferation, differentiation, and survival of B-cells, and include both antigen presentation and humoral signals (cytokines like IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN-γ) [151]. Several pairs of other receptor-ligand molecules are also involved, including the CD40-CD40 ligand (CD40L) in B-cell immunoglobulin isotype switching, and others like B7/CD28, CD11a (LFA-1)/CD54 (ICAM-1), and CD58 (LFA-3)/CD2 in T- and B-cell activation [151]. Only about 30% of naïve neonatal T-cells express CD40L, compared to 80% of the naïve T-cells from adults [152]. However, once primed with mitogen(s) and IL-2, which converts naïve T- cells to the memory phenotype, neonatal T-cell CD40L expression is upregulated to adult levels [153]. The capacity of neonatal B-cells to differentiate into plasma cells and undergo isotype switching can be variable (*vide infra*), but in general, neonatal T-cells are less efficient at providing humoral and CD40-dependent activation signals [154, 155].

108.4.5 Natural Killer Cells

108.4.5.1 Development

NK cells share some T-cell markers, but are not affected in many natural/experimental disruptions of the T-cell system [156–158]. It is conceivable that the two lineages derive from a common progenitor [159, 160]. Morphologically, NK cells are large granular lymphocytes, and have characteristic surface markers including the CD56/neural cell adhesion molecule and CD16/$F_c\gamma$ receptor IIIa ($F_c\gamma$RIIIa), a low affinity IgG receptor. They also express CD2, LFA-1 and cytokine receptors such as IL-2$R_{\beta\gamma c}$, IL-12R, IFN-γR and IL-15R_α [161].

NK cells can be detected as early as 6 weeks of gestation, and the number then increases progressively until birth. In cord blood, 10–15% of all lymphocytes are NK cells, which is comparable to adult peripheral blood [159]. The phenotype of fetal NK cells, however, is different from that of adults' cells. Fifty to 80% of fetal NK cells express CD3γ, ε, λ, and σ proteins, unlike a much smaller number in term infants, or in adults where only CD3σ is expressed [159]. On the other hand, only 30–50% of the fetal NK cells express CD16 (compared with more than 90% of neonatal and adult NK cells). Similarly, CD56 and CD57 are expressed poorly on fetal or neonatal NK cells, compared to nearly 50 percent positivity in adult NK cells [159, 162].

108.4.5.2 Function

NK cells recognize viral-infected and tumor cells by the absence or decreased expression of MHC class I molecules on the cell surface [163]. Their MHC-unrestricted killing is mediated by perforin/granzyme apoptotic pathways [164]. The other mechanism of cytolysis is antibody-dependent cell-mediated cytotoxicity (ADCC), where target cell-bound IgG1 or IgG3 triggers the $F_c\gamma$RIIIa receptor on the NK cell [163, 165]. NK cells are also believed to play a key role in maintaining immunological tolerance at the maternal-fetal interface [166].

Fetal NK cells have a significantly lower cytolytic activity (including ADCC) against tumor cell target cell lines than adults, but it increases with gestational age parallel to increasing expression of CD56 and CD16 [153, 159]. However, even at term, the cytolytic activity is only 50–80% of adult levels [160].

NK cells should not be confused with the natural killer T-cells, a heterogeneous group of T-cells that express an alpha beta T-cell receptor along with some of the NK cell markers. Many of these cells recognize the non-polymorphic CD1d molecule, an antigen-presenting molecule that binds self- and foreign lipids and glycolipids. NK T-cells constitute only 0.2% of all peripheral blood T-cells. These cells play an important role in mucosal immunity and in the pathogenesis of inflammatory/allergic conditions; the role during fetal life remains unclear [167]. Imbalances in this system produced by chronic inflammation may be involved in the presentation of the acquired forms of hemophagocytic lymphohistiocytosis in the neonatal period.

108.5 The Mucosal Immune System

108.5.1 Peyer's Patches and Other Organized Lymphoid Tissue

Peyer's patch anlagen become identifiable in fetal ileum at 11 weeks as aggregates of HLA-DR^+, $CD4^+$ lymphoid cells [168, 169]. Major events in Peyer's patch development have been summarized in Fig. 108.3 and Table 108.1 [169–171]. At birth, the organized lymphoid compartment is naïve but structurally complete, and the predominant activity involves proliferative expansion (rather than primary lymphopoiesis) [171]. The number of PP increases from about 60 at birth to over 200 by 12–14 years [172].

In the vermiform appendix, the development of lymphoid structures lags behind the Peyer's patches [173]. Appendiceal

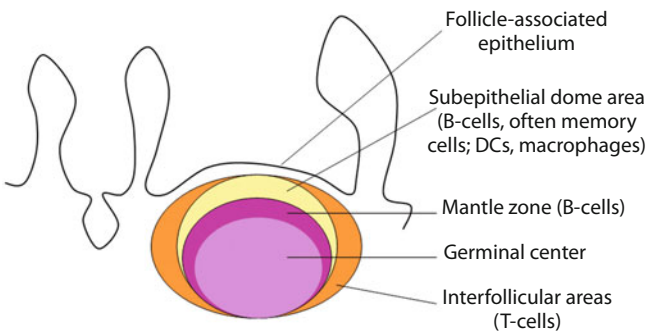

Fig. 108.3 Structure of Peyer's patch

Table 108.1 Development of Peyer's patches in the human fetus

11 wks gestation	PP anlagen with HLA-DR+ CD4+ lymphoid cells
16 wks gestation	Appearance of T- and B-cells First appearance of CD8+ cells* B-cell maturation with appearance of surface IgM and IgD
16–18 wks gestation	Appearance of CD5+ B-1 cells Surface IgA on B-cells
18–20 wks gestation	Appearance of PP zonation into B- and T-cell areas
24 wks gestation	PP are macroscopically identifiable
0–4 wks postnatal	Germinal center formation

* Fetal Peyer's patch T-cells are predominantly of the $CD4^+$ phenotype.

lymphoid follicles enlarge rapidly after birth following bacterial colonization and translocation [174]. The first IgA^+ plasma cells appear at 2 weeks after birth and then increase to adult levels at 4–5 months. To what extent full and appropriate capacity of this system contributes to the susceptibility of the preterm neonate to the development of necrotizing enterocolitis remains to be ascertained.

108.5.2 Lymphocytes in the Lamina Propria and Intra-Epithelial Compartments

Scattered B-cells are first observed in the lamina propria at 14 weeks gestation [169]. The fetal intestinal B-cell population consists of two distinct cell types. The first population of large, dividing, mature B-cells shares morphologic and phenotypic ($CD20^+IgM^+IgD^+$light $chain^+$) features with the thymic B-cells. These are large-sized cells with extensive cytoplasmic processes, which are in contact with adjacent T-cells. A second population of smaller pre-B-cells (IgM^+ light $chain^-$ $CD20^-$) has also been identified, suggesting the presence of local B-cell development [175]. As extrathymic T-cell development occurs in human fetal intestine (*vide infra*), it has been hypothesized that as in the thymus, the B-cells may play a role in the development and selection of the T-cells.

The B-cell population in the fetal intestine comprises IgM^+ and IgG^+ cells [176]. The fetal intestinal B-cell repertoire is similar to B-cells in circulation or other organs, but differs significantly from plasma cells in postnatal intestine [175]. After birth, the IgM^+ plasma cell population expands faster than IgG^+ cells, and at the same time microbial stimulation induces B-cells to undergo IgA class switch in both the lamina propria and organized lymphoid tissue [177]. IgA+ plasma cells are first seen in the lamina propria during the second postnatal week [178]. The number of IgA^+ cells in the mucosa reach adult levels at 2 years, although serum IgA concentrations reach adult levels only during the second decade [170].

Intestinal T-cells can be identified from 12–14 weeks of gestation [179]. Outside the organized lymphoid tissue, intestinal T-cells are distributed as intra-epithelial (IELs) and lamina propria lymphocytes (LPLs). The fetal gut has a small number of IELs (3–5 $CD3^+$ IEL/100 IECs compared to 6–27 cells/100 IECs in older children), which expand rapidly after birth (a 10-fold expansion of the αβ T-cells and a 2–3 fold increase in the γδ cells, vide infra) [170, 180]. In contrast, LPLs continue to expand during fetal period and have a density similar to the postnatal intestine by 19–27 weeks gestation [179].

Several early lineage T-cell populations can be seen in the fetal intestine, suggesting that T-cells may develop locally in an extrathymic pathway [169, 171, 179, 181]. These immature T-cell lineages are shown in Fig. 108.4. Whereas most immature LPLs differentiate rapidly after birth, the differentiation of IELs is slower and continues through infancy [182]. In addition to phenotypic changes, intestinal T-cells also continue to undergo functional maturation during infancy and childhood. The TCR β-chain repertoire is polyclonal during fetal period and infancy and only gradually becomes restricted to the oligoclonal pattern characteristic of adults. This restriction is likely due to expansion of a few dominant clones, which are specific for the commensal bacterial flora [182].

In the fetal intestine, about 10–30% of IELs express the γδ T-cell receptor [169]. Rodent studies suggest that γδ cells may regulate IEC function, display cytotoxic activity and may promote antimicrobial immunity [183, 184]. Similar to αβ T-cells, the fetal/neonatal γδ repertoire is also polyclonal [185].

108.5.3 Secretory Immunoglobulins

Secretory immunoglobulins, IgA and IgM, play an important role in mucosal defense. Secretory IgA (sIgA) can be detected in mucosal secretions as early as 1–8 weeks after birth [186–189]. sIgM, on the other hand, appears transiently during early infancy [188].

sIgA levels rise during the neonatal period to reach an initial peak (as measured in saliva) at 4–6 weeks. In premature infants, sIgA appears in secretions at a similar chronological age as in full-term infants, although sIgA concentrations may be lower. If chronological age is corrected for prematurity, sIgA concentrations then become similar to matched full-term infants [190, 191]. Salivary IgA levels continue to rise up to 18 months of age [191]. A transient nadir in sIgA has been inconsistently [189, 192] recorded at 3–6 months [188, 193].

Secreted immunoglobulins also change qualitatively during the first year. There is a switch from monomeric IgA to polymeric sIgA sometime during the first year, indicating maturation of the secretory immune system [194], or alternatively, increasing exposure to exogenous antigens [195]. The relative amounts of IgA subclasses in mucosal secretions also changes during infancy. At birth, sIgA1 is the dominant subclass but sIgA2 increases rapidly by 6 months of age [192].

Specific sIgA responses appear to be related more to the timing and quantum of the antigenic stimulus than to developmental factors during infancy. sIgA antibodies to *E. coli* somatic antigens appear in neonates within a few weeks after timed exposure and colonization [196]. The strength of the stimulus also has an effect: earlier, and stronger, specific sIgA responses are seen in neonates born in areas endemic for a pathogen compared to infants in the developed world [194, 197].

During the neonatal period, colostrum provides an important alternative source of sIgA [198]. Milk antibodies, amounting to about 0.5–1 g/day throughout lactation (comparable to the 2.5 g/day being produced by a 65 kg adult), are directed against antigens present in the environment shared by the mother-infant dyad [199]. The presence of enteromammary and bronchomammary pathways allow immune cells stimulated by antigens in the maternal intestine and bronchial mucosa to migrate to the mammary gland [198, 200]. Interestingly,

sIgA levels have been reported to be higher in colostrum and milk of mothers of preterm neonates [201].

Premature infants lack the intrinsic protective mechanisms of the adult intestinal mucosa that prevent sensitization against luminal constituents: a strong physical barrier, luminal enzymes that can alter ingested antigens, presence of regulatory T-cells, and the production of sIgA [202]. The risk of sensitization is further increased due to several developmental deficiencies within primary immune cells: (1) specific antibody responses in premature infants are abnormal due to reduced antigen affinity, increased polyreactivity, and autoreactivity [147, 203]; (2) the lengths of immunoglobulin heavy chain third complementarity determining regions (CDR3) are almost 3 amino acids shorter in the fetus/premature infant than adults [204]. This reduces the potential antibody diversity available to the fetus/preterm neonate by about 20^3 (= 8000) fold [204]. Moreover, antigen binding sites with short CDR3 regions, due to their tertiary structure, are more likely to bind to peptides such as allergens [205]; and (3) the short CDR3 regions of fetal $CD5^+$ B1 cells share characteristics with variable regions of IgE heavy chains [206, 207]. These observations have led to the hypothesis that B1 cells may contribute to the repertoire of allergen specific IgE^+ plasma cells, and that premature exposure of the immature intestinal B-cell repertoire to allergens may influence the risk of sensitization [207].

108.5.4 Intestinal Macrophages and Dendritic Cells

Macrophages first appear in the developing intestine at 11–12 weeks of gestation, increase rapidly during the 12–22 week period, and then continue to expand at a slower pace through early childhood [176, 208, 209]. These cells play a critical host defense role in being the first phagocytic cells of the innate immune system to encounter luminal bacteria that breach the epithelium and gain access to the lamina propria [60, 210]. Intestinal macrophages display avid phagocytic and bacteriocidal activity but are markedly attenuated in their inflammatory responses [60], a unique adaptive mechanism that prevents unnecessary inflammation in the gut mucosa despite the proximity to luminal bacteria. In sick and preterm neonates who are predisposed to bacterial translocation due to an abnormally permeable gut epithelial barrier, immaturity of the local adaptive immune system and low secretory IgA production [174, 211], intestinal macrophages assume even greater importance as a host defense system because of their ability to eliminate previously unknown bacteria through phagocytosis and intracellular killing. A breach of the gut mucosal barrier defense is met by tissue macrophages in the liver (Kupffer cells).

Intestinal macrophages are derived from circulating monocytes, which are recruited to the mucosa under the influence of various epithelial- and mesenchymal cell-derived chemoattractants [60, 210, 212]. Because neither intestinal macrophages nor their precursor monocytes have the ability to undergo clonal expansion [210], the only mechanism available for the development and maintenance of the gut macrophage pool is through the continuous recruitment and differentiation of blood monocytes. In adults, interleukin-8/CXC ligand 8 (IL-8/CXCL8) and transforming growth factor-beta (TGF-β) recruit macrophage precursors to the intestinal mucosa [210]. However, several lines of evidence indicate that IL-8 and TGF-β may not be important as macrophage chemoattractants in the fetal intestine. In the fetus, IL-8 is mainly comprised of a longer, less-potent 77-amino acid isoform (unlike the shorter 72-amino acid isomer in the adult) [213]. Similarly, TGF-β bioactivity is low in the early-/mid-gestation fetal intestine. Finally, macrophages appear in the fetal intestine at least a few weeks before lymphocytes or neutrophils [208, 209], suggesting that macrophage precursors are likely to be recruited to the early fetal intestine by chemoattractant(s) more specific for macrophage precursors than IL-8/CXCL8, which recruits both neutrophils and macrophage precursors [20, 210], or TGF-β, which mobilizes macrophage precursors as well as T lymphocytes [210, 214]. We have shown recently that epithelial cells in the fetal intestine produce chemerin (previously known as tazarotene-induced gene-2/TIG2 or retinoic acid receptor responder-2/RARRES2), to recruit macrophage precursors.

We have shown recently that unlike in the adult, intestinal macrophages in the midgestation fetus/premature infant are responsive to bacterial products and produce inflammatory cytokines. This inflammatory downregulation of fetal intestinal macrophages occurs under the influence of TGF-β, particularly the TGF-β_2 isoform. Further investigations are currently in progress to determine whether the incompletely-developed macrophage tolerance to bacterial products in the preterm intestine could predispose these infants to necrotizing enterocolitis.

There is very limited data on fetal/neonatal intestinal DCs [215]. $HLA\text{-}DR^+$ DC-like cells can be detected in both the lamina propria as well as the Peyer's patches after 14 weeks, but these cells may have some overlap with lamina propria macrophages. In rats and non-human primates, DCs have been noted in the fetal lamina propria as well in Peyer's patches [216]. The functional importance of these DCs is not clear.

108.6 Conclusions

It is apparent that the extraordinary complexity of the human immune system in general is amplified at the time of birth by the fetal stage of development, the postnatal age when examined, the ontological capacity of stem cells to respond to

maturational signals and each issue is compounded by the transition from a Th2 to a Th1 phenotype at the conclusion of pregnancy. All of these factors are occurring in tandem with a first inoculum of maternal bacteria as well as her repertoire of related antibodies followed by the acquisition of subsequent bacterial colonizers/modifiers of gut and skin flora (as influence by antibiotic exposure or ex utero environments) and nutrient-derived antigens that must all be distinguished from self to ensure an effective barrier defense and nutrient absorption. It is perhaps more surprising that this system works at all than the fact that there are limitations that increase vulnerability of neonates to infection. A fundamental and expanded understanding of these relationships will further the hope of identifying opportunities to augment immune defenses in ELGANs where bacterial invasiveness ranges from 30–50% of births.

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Congenital Immunodeficiencies

Alessandro Plebani and Gaetano Chirico

109.1 Introduction

The primary immunodeficiency diseases (PIDs) are a heterogeneous group of inherited disorders with defects in one or more components of the immune system. PIDs are classified into T lymphocyte, B lymphocyte, phagocytic cell, and complement deficiencies. This classification is simple and practical because clinical symptoms, mainly susceptibility to infections, vary according to the affected arm of the immune system. The prognosis of these disorders and their treatment depends on their early recognition and initiation of appropriate management.

Evaluation of immune function should be considered for children with clinical symptoms of a distinct immune disorder or with unusual, chronic, or recurrent infections, such as: one or more systemic bacterial infections (sepsis, meningitis); two or more serious bacterial infections (cellulitis, suppurative otitis media, pneumonia, lymphoadenitis) during the first year; serious infections occurring at unusual sites (liver, brain abscess, omphalitis); infections with opportunistic pathogens (*Aspergillus*, *Serratia marcescens*, *Pneumocystis jiroveci* (formerly *P. carinii*), *Cryptosporidium*, *Nocardia*); infections with common childhood pathogen but with unusual severity.

Clinical suspicion of a primary immunodeficiency disease should be raised in case of a history of consanguinity or a positive family history for a congenital immunodeficiency. Physical signs include an absence of lymphoid tissue or the presence of syndromic features. Severe or recurrent infections caused by encapsulated bacteria suggest a primary B-cell or complement defect, and they occur after the first 6-12 months of age, in parallel with the decrease of the maternally acquired IgG. On the contrary, severe viral (adenovirus, cytomegalovirus, respiratory syncytial virus), fungal or opportunistic infections during the first few months of life, are indicative of a primary T-cell defect.

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A complete differential blood count and assessment of immunoglobulin serum levels are the most cost-effective diagnostic screening tests. A normal neutrophil count excludes neutropenia, and normal platelet size or counts exclude the Wiskott-Aldrich syndrome. If the absolute lymphocyte count is normal, the patient is unlikely to have a severe T-cell defect because T-cells normally represent 70% of circulating lymphocytes and their absence results in severe lymphopenia. This is not the case for T^-B^+ severe combined immunodeficiencies, in which the presence of circulating B-cells results in a normal absolute lymphocyte count and cytofluorimetric analysis of B- and T-cell subsets reveals that virtually all lymphocytes belong to the B-cell lineage. Low immunoglobulin serum levels with normal T-cell counts indicates a diagnosis of a primary B-cell defect. Normal neutrophil, T- and B-cell counts should point to a functional defect of phagocytes. The genes responsible for many PIDs are known, which may lead to successful neonatal screening programs. In this chapter we will focus on primary T- and B-cell defects and related disorders.

109.2 Defects of the B Cell Compartment

109.2.1 Agammaglobulinemia and Common Variable Immunodeficiency

Agammaglobulinemia, X linked (XLA or Bruton's Agammaglobulinemia) or autosomal recessive agammaglobulinemia (ARA) represent the prototype of disorders of the B-cell compartment. Both disorders are characterized by absent/low immunoglobulin serum levels, defective antibody response to antigens, and absence of circulating B-cells in the presence of normal T-cell counts and function. Both disorders result from an early block of B-cell development, caused in XLA by mutations in the gene encoding Bruton's tyrosine kinase (Btk), which has a crucial role in early B-cell differentiation. XLA accounts for 70–90% of agammaglobulinemic patients. Five

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distinct autosomal recessive genetic defects have been found to cause ARA, including mutations in the genes encoding the following: the μ heavy chain; Igα and Igβ signalling molecules; B-cell linker adaptor protein (BLNK); the surrogate light chain, λ5/14.1; and in the gene leucine-rich repeat-containing 8 (*LRRC8*). With the exception of *BLNK* and *LRRC8*, the other genes encode for components of the B-cell receptor complex, which is responsible for transducing the signals necessary for early B-cell development [1]. The onset of symptoms in agammaglobulinemia usually occurs early in infancy between 6 and 12 months as maternally acquired IgG decrease. Infections may start sooner in extremely preterm infants. A positive family history of primary immunodeficiencies is an indication for immunological investigation even in the absence of clinical signs. Bacterial infections are the most common clinical manifestations of agammaglobulinemia, usually by *Streptococcus pneumoniae*, *Haemophylus influenzae*, *S. aureus* and *Pseudomonas* species. Infections include otitis media, sinusitis, pneumonia, sepsis and meningitis. Less common bacterial species, such as *Salmonella* and *Campylobacter* account for gastrointestinal infections. Viral infections are usually handled normally with the exception of hepatitis viruses and enteroviruses [1]. Live attenuated vaccines should not be administered. Physical examination often reveals lymphoid hypoplasia with minimal or no tonsillar tissue and no palpable lymph nodes. Patients found to have low/absent immunoglobulin serum levels should have their B-cells evaluated by flow cytometry using monoclonal antibodies against CD19 or CD20. Normally, approximately 10% of circulating lymphocytes are B-cells. B-cells are absent (or <1%) in both XLA and ARA. However, hypogammaglobulinemia in the presence of normal B-cell counts suggests a diagnosis of common variable immunodeficiency (CVID). This distinction is important because children with hypogammaglobulinemia due to XLA/ARA or CVID can have different clinical problems, and the two conditions clearly have different inheritance patterns. Although CVID is considered a disorder of adults, it may also occur in infancy.

Because of the transient hypogammaglobulinemia of infancy, a definite diagnosis of CVID cannot be made early in life unless a defective antibody response to antigens is documented. Gene sequence analysis of the genes causing CVID (*ICOS*, inducible co-stimulator; *TACI*, transmembrane activator, calcium modulator, and cyclophilin ligand interactor; *BAFF-R*, B-cell activating factor of the TNF family receptor, *CD19*, *CD20* and *CD81*), is useful for providing a definitive early diagnosis. However, mutations of these genes account only for 10% of CVID patients [1, 2].

Immunoglobulin replacement therapy is the treatment of choice in agammaglobulinemic patients. Current protocols are based on intravenous immunoglobulins (IVIG) or subcutaneous immunoglobulins (SCIG). Several international studies have shown that maintaining pre-infusion IgG levels > 500 mg/dL results in a notable reduction in the number of infections. This protective IgG level is usually achieved by administration of 400 mg/kg every 3–4 weeks.

109.3 The Hyper IgM Syndrome (HIGM)

HIGM is a heterogeneous group of genetic disorders characterized by normal/elevated IgM associated with low IgG and IgA serum levels because of a defect in the immunoglobulin class switch recombination process. There are X linked and autosomal recessive forms of the disorder [3, 4]. The X-linked forms are caused by mutations of the *CD40 Ligand* (HIGM1) or *NEMO* (nuclear factor κB essential modulator) genes. The autosomal recessive forms are due to mutations of the *AID* (activation-induced cytidine deaminase, HIGM2), *UNG* (uracil DNA glycosylase), or of the *CD40* gene (HIGM3). Distinct clinical features are associated with different genetic defects. HIGM1 and HIGM3 are the most severe forms and their clinical presentation is similar to that observed in immunodeficiencies characterized by T-cell defects. In fact, CD40L is expressed on activated CD4 T-cells and CD40 on B and dendritic cells and defective expression of these molecules ultimately leads to a defect of T-cell priming. Most patients present in infancy with bacterial infections of the upper and lower respiratory tract and have a unique predisposition to *Pneumocystis jiroveci* pneumonia (PCP). These patients are also susceptible to infection with opportunistic pathogens, such as Cytomegalovirus, *Histoplasma capsulatum*, *Cryptococcus*, and *Cryptosporidium parvum*, which is associated with chronic diarrhea. Infection of the biliary tree with Cryptosporidium parvum, may predispose patients to sclerosing colangitis and to tumor of the liver, pancreas or biliary tree. Neutropenia is a common finding in HIGM1. The HIGM caused by the *NEMO* gene is easily recognized because of the presence of anhydrotic ectodermal dysplasia with sparse scalp hair, conical teeth and absent sweat glands.

The HIGM syndromes due to mutations of AID and UNG genes are considered primary B-cell defects. There is typically an increased susceptibility to bacterial infections, similar to agammaglobulinemic patients often with lymphoid hyperplasia.

In the case of low/absent levels of serum IgG and IgA with normal/elevated IgM, the diagnosis of HIGM1 is usually made by demonstrating an inability of activated CD4+ T-cells to express functional CD40L. HIGM2 is diagnosed by demonstrating B-cells that are unable to express the CD40 molecule. Diagnosis of HIGM caused by mutation of AID or UNG genes requires gene sequence analysis.

Except for CD40L and CD40 deficiency, for which stem cell transplantation is recommended [3], appropriate use of antibiotics to treat infections and the regular administration of intravenous immunoglobulins are the only effective treatments for these disorders.

Patients with CD40L and CD40 deficiency should be given co-trimoxazole as prophylaxis for *Pneumocystis jiroveci* pneumonia, advised to drink boiled water, and azithromycin prophylaxis may lessen the risk of *Cryptosporidium parvum* infections.

109.4 Defects of the T Cell Compartment

109.4.1 Severe Combined Immunodeficiencies (SCIDs)

SCIDs are a heterogeneous group of congenital disorders characterized by block of T-cell differentiation, variably associated with abnormal development of other lymphocyte lineage, such as B or natural killer (NK) cells [5]. The overall frequency of these disorders is estimated to be 1: 50,000–1:100,000 live births. SCIDs include a large number of disorders with X-linked or AR-inheritance, most of which are now molecularly defined. The clinical presentation is quite uniform, among the various SCIDs. Affected patients present within the first few months of life with infections, mainly of the respiratory and gastrointestinal tract. Oral candidiasis, persistent diarrhea with growth impairment and/or interstitial pneumonitis are the most frequent manifestations. These patients have increased susceptibility to infections caused by opportunistic organisms including *Candida albicans*, *Pneumocystis jiroveci* and *Aspergillus* species or by viruses (adenovirus, cytomegalovirus, respiratory syncytial virus). Live attenuated vaccines are another cause of severe clinical manifestations. BCG vaccination may lead to disseminated, often lethal infection; progressive central nervous system poliovirus infection can occur secondary to oral polio vaccination or exposure to a recently vaccinated individual.

Non-infectious clinical manifestations consist mainly of graft-versus-host disease (GVHD), presenting with a severe skin rash, caused by maternal engraftment or following transfusion with non-irradiated blood derivatives.

A diagnosis is possible at birth, with most affected infants having lymphopenia (less than 2000 lymphocytes/mm^3). Lymphocyte phenotyping shows a low number of T lymphocytes. B lymphocytes and NK cells may be present or absent depending on the type of SCID. If a high T-cell count is against a diagnosis of SCID, but there are typical clinical symptoms, a systematic search for the maternal origin of the circulating T-cells should be performed. Serum immunoglobulin concentrations are diminished or absent and no antibody response can be elicited after immunization. Four lymphocyte phenotypes are possible on the basis of the influence of the defective gene on T-cell, B-cell, and NK cell development. The $T^-B^+NK^+$ immunophenotype is due to CD3δ–, or CD3ε–, or CD3ζ, or interleukin-7 receptor α-chain deficiency, the $T^-B^+NK^-$ form includes the X-linked SCID which is due to γc deficiency, and the autosomal recessive forms due to JAK3 (Janus kinase 3), or CD45 deficiency, the $T^-B^-NK^+$ form is caused by RAG1, RAG2 (recombination-activating gene), or Artemis deficiency, the $T^-B^-NK^-$ form is caused by ADA (adenosine-deaminase) deficiency. A total lack of both lymphocytes and granulocytes, is suggestive of reticular dysgenesis which is due to mutations of *AK2* (adenylate kinase 2).

The natural course of SCIDs is severe, with most patients dying within the first years of life, unless properly treated. Supportive therapy, which may at best prolong survival, consists of intravenous immunoglobulins, aggressive treatment of infectious episodes, prophylactic co-trimoxazole (to prevent *Pneumocystis jiroveci* pneumonia), and irradiated blood products. Bone marrow transplantation (BMT), or other stem cell transplantation, often results in permanent cure, with a survival rate of 90% if an HLA-identical family donor is available. Excellent results (80% survival) have been obtained with HLA-matched unrelated donor and with aploidentical donor (75% survival). ADA-deficient SCID and X-linked SCID have been treated with somatic gene therapy; although serious adverse events occurred in the case of X-SCID. ADA-deficient SCID is also managed with regular injections of polyethylene glycol conjugate to the bovine-derived adenosine deaminase (PEG-ADA).

109.4.2 Combined Immunodeficiencies (CID)

CID is distinguished from SCID by the presence of low but not absent T-cell function. Similar to SCID, CID is a syndrome of various genetic defects. Clinical presentation may overlap with that of SCID patients, although sometimes infections develop slightly later. CID is an heterogeneous group of immune disorders among which the following are included.

109.4.2.1 MHC Class II Antigen Deficiency

MHC class II antigen deficiency is an autosomal recessive primary immunodeficiency caused by the absence of MHC class II expression on cells normally expressing HLA class II molecules [6]. This disorder is caused by mutations in several different genes, which code for a complex of regulatory factors controlling transcription of MHC II genes. MHC class II-deficient patients have a very low number of CD4 T-cells but normal or elevated numbers of CD8 T-cells. Lymphopenia is only moderate. Patients are hypogammaglobulinemic due to impaired antigen-specific responses caused by the absence of antigen-presenting molecules. These patients present, from early infancy, with an increased susceptibility to viral, bacterial, fungal, and protozoan infections, primarily of the respiratory and gastrointestinal tract. Curative treatment is BMT, although it has limited success in comparison with other types of T-cell immunodeficiencies.

109.4.2.2 Wiskott-Aldrich Syndrome

The Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by microplatelet thrombocytopenia, eczema, recurrent infections, and increased risk of autoimmunity and lymphoreticular neoplasia [7]. Thrombocytopenia and small platelet volume in a male should raise suspicion of the diagnosis. This disorder is caused by mutation of the *WASP* (WAS protein) gene. *WASP* is expressed in

all hematopoietic cells, including CD34+ stem cells. Mutation of this gene not only cause WAS, but also X-linked thrombocytopenia (XLT), a milder form of the disease characterized by microplatelet thrombocytopenia, but without the clinical findings associated with the classic WAS phenotype. The classic clinical symptoms (bleeding, infections and eczema) are usually not present simultaneously. The earliest manifestation, often present at birth, consists of petechiae and bruises. Additional early manifestations of thrombocytopenia include bloody diarrhea, and hemorrhage following circumcision. Eczema and recurrent bacterial infections usually develop during the first year of life; later, infections with microorganisms such as *P. jiroveci*, and the herpes viruses become more frequent. The severity of the immune deficiency can vary depending largely on the type of mutation and its effect on protein expression. Both T and B lymphocyte functions are affected. During infancy the number of circulating lymphocytes might be normal or moderately decreased. Consistent findings are low isohemagglutinins titers, and marked decreased response to polysaccharide antigens.

Intravenous immunoglobulin, appropriate treatment and control (use of killed vaccines) of infections and eczema, platelet transfusion for serious bleeding episodes are part of an appropriate supportive therapy. Bone marrow transplantation is the treatment of choice and may be curative.

109.5 Immunodeficiencies as Part of Complex Diseases

109.5.1 DiGeorge Syndrome (DGS)

DGS is one of the most common chromosomal disorders known (estimated prevalence of 1/4000–1/6000 persons). It is caused by developmental defects in the third pharyngeal pouch and fourth pharyngeal arch [8] and defects are found in the thymus, heart and parathyroid glands. Approximately 90% of patients with DGS are hemizygous for 22q11; in rare instances, patients are hemizygous for 10p13. DGS is characterized by the triad of clinical features: congenital heart defects, immunodeficiency secondary to thymic hypoplasia, and hypocalcemia secondary to parathyroid gland hypoplasia. However, it is now well recognized that the phenotypic features of DGS are much more variable and extensive than previously recognized and frequently overlapping with other disorders including velocardiofacial syndrome (VCFS) and conotruncal anomaly face syndrome (CTAFS), both of which are frequently associated with 22q deletions. The collective acronym CATCH22 syndrome (Cardiac defects, Abnormal facies, Thymic hypoplasia, Cleft palate, and Hypocalcemia resulting from 22q11 deletions) has been proposed for these differing presentations, but should probably be avoided when considering individual patients. Dysmorphic facial features of DGS include: hypertelorism, low-set, prominent ears, micrognathia, high arched palate, short philtrum of the upper lip. Cardiac defects, dysmorphic facial features and hypocalcemic seizures occurring during the neonatal period should raise the suspicion of DGS.

All children suspected of DGS should have an immunological work up. Most infant with DGS have a mild and transient immunodeficiency. Immunoglobulin serum levels are usually normal, T-cell production is usually moderately impaired and T-cell mitogen responses are often normal or near normal. These patients usually develop normal or near-normal immunologic function over time. Total thymic aplasia is present in less than 1% of cases. It occurs in complete DGS, when patients resemble SCID in their immunological phenotype and susceptibility to infections with low grade or opportunistic pathogens. Bone marrow and thymus transplantations have been performed in complete DGS.

109.5.2 Cartilage-Hair Hypoplasia (CHH)

CHH is a rare autosomal recessive disorder characterized by chondrodysplasia with growth failure, hypoplastic hair, defective immunity and erythrogenesis. CHH is caused by mutations in the *RMRP* (ribonuclease mitochondrial RNA-processing) [9]. The disease is prevalent among the Old Order Amish in the United States and in the Finnish population. Growth failure, has its onset prenatally, and is usually due to short limbs. All segments of the limbs are affected. The major radiological abnormalities are confined to the methaphyseal parts of the tubular bones, which are flared, scalloped, and irregularly sclerotic. The majority of the patients have sparse, fine, and silky hair. Hirschsprung's disease and predisposition to malignancies have been reported.

The defective cellular immunity is characterized by mild to moderate lymphopenia and impaired *in vitro* lymphocyte response to mitogens. Humoral immunity is usually intact. In a few patients a more severe immunological phenotype, similar to combined immunodeficiency (CID), has been reported. Deficient erythrocyte production presents usually as mild macrocytic anemia in early childhood, with spontaneous recovery before adulthood. Patients occasionally present with severe congenital hypoplastic anemia.

Hematopoietic cell transplantation has resulted in successful immune-reconstitution in CHH patients who present with CID, but will not affect the morphologic features or dwarfism.

109.5.3 Nijmegen Breakage Syndrome (NBS)

NBS is an autosomal recessive genetic disease belonging to a group of disorders often called chromosome instability syndromes, characterized by microcephaly, particular "bird-like" face, growth retardation, immunodeficiency and predisposition to cancer [10]. NBS is caused by mutation of the *NBS* gene, which encodes for a protein involved in the repair of DNA double-strand breaks. Suspicion of NBS should be

raised by intrauterine growth restriction, microcephaly and facial dysmorphism (sloping forehead, receding mandible, prominent mid face, upward slant of the palpebral fissures). Chromosomal rearrangements, typically involving chromosomes 7 and 14, and chromosomal hypersensitivity to X-irradiation are useful laboratory tests to support the clinical suspicion. The definitive diagnosis is through gene sequence analysis: the 657del5 mutation is present in approximately 90% of cases. Hypogammaglobulinemia is common. Mild to moderate lymphopenia is present with an impaired *in vitro* lymphocyte proliferative response to mitogens. The primary cause of death is cancer, with a median of age of cancer-related death of only 10 years. Patients also develop recurrent upper and lower respiratory tract infections and chronic lung disease is the second leading cause of death. Differential diagnosis should include ligase-4 deficiency, which shows overlapping clinical and laboratory findings with NBS. Ataxia telangiectasia (AT), overlaps NBS in a number of characteristics, including chromosomal instability, radiosensitivity, and cancer predisposition, but AT patients do not display the characteristic "bird-like" facial appearance, microcephaly and growth retardation of patients with NBS.

109.5.4 Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX)

IPEX is a rare genetic disorder of immune regulation caused by mutations in the *FOXP3* gene located in the centromeric region of the X chromosome [11]. The product of this gene is required for the development of CD4+CD25+ T regulatory cells. In the absence of T regulatory cells, activated CD4+ T-cells produce multi-organ damage resulting in autoimmune manifestations such as type I diabetes mellitus, severe enteropathy, hypothyroidism, and autoimmune skin disease resembling eczema, psoriasis, or atopic dermatitis. These clinical manifestations, often fatal, usually occur in early infancy and often appear sequentially, rather than simultaneously. Immunologic testing reveals little beyond variably increased IgE levels, eosinophilia, mild increase in CD4:CD8 ratio and an increase in T-cell activation markers. Autoantibodies are often absent early but develop gradually or acutely. Immunosuppressive drugs have been used with some success. Unfortunately, these drugs do not maintain long-term remission of symptoms, may be toxic and facilitate opportunistic infections. Hematopoietic stem cell transplantation may be considered for the most severe forms.

109.5.5 Autoimme Polyendocrinopathy Candidiasis Ectodermal Dystrophy Syndrome (APECED)

APECED, also known as APS-1 (autoimmune polyglandular syndrome type 1), is an autosomal recessive disorder due to mutation of the *AIRE* (autoimmune regulator) gene [11]. The product of this gene is crucial for the induction of central tolerance through the regulation of self-antigen gene expression in the antigen presenting cells of the thymic medulla. Thus, defects of *AIRE* lead to a defective selection of organ-specific T-cells, which are responsible for autoimmune manifestations. APECED is characterized by a set of three abnormal features: chronic mucocutaneous candidiasis, hypothyroidisms and adrenal insufficiency. At least two of these major components need to be present for diagnosis. Usually the first sign of the syndrome is mucocutaneous candidiasis, which may occur soon after birth, while other signs appear later. High serum antibody titers to components of the affected endocrine organs are characteristic. Current management is supportive.

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Inflammatory Mediators in Neonatal Asphyxia and Infection

Marietta Xanthou and Victoria Niklas

110.1 Introduction

Inflammatory mediators produced in response to hypoxic-ischemic (H-I) injury and infection in the newborn, include multi-potent cytokines and chemokines released by a variety of somatic and bone marrow-derived cells both locally, in the brain and systemically, in the circulation. Pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-18, interferon (IFN)-γ and tumor necrosis factor (TNF)-α, are small polypeptides secreted in response to cellular injury and inflammation. Cytokines trigger somatic and immune cell activation, differentiation and death after signaling through their cognate receptors. Chemokines, such as CCL2 and CXCL8, are chemotactic cytokines that induce cells of the innate and adaptive immune system to leave the blood stream and migrate to sites of injury in the central nervous system (CNS) and in the periphery [1]. Cytokines and chemokines are considered critical mediators of brain damage and clinical indicators of overwhelming sepsis in the newborns. We review the cellular origin and role of cytokines and chemokines in these diseases. We also discuss treatment modalities that may interrupt these inflammatory cascades. A better understanding of the role of cytokines and chemokines in response to H-I injury and infection is likely to improve the outcome and long-term prognosis of these diseases in the newborn period.

110.2 Inflammatory Mediators and White Matter Injury in the Newborn

Hypoxia, ischemia and infection result in acute inflammatory responses and significant brain injury of the newborn. Moreover, preterm and low birth weight (LBW) infants may be at increased vulnerability due to cerebrovascular immaturity. White matter injury in the brain is a major cause of neurodevelopmental impairment and long-term disability in premature LBW infants [2]. Indeed, CNS injury following H-I injury and infection, as well as, their interplay with inherent vascular and oligodendroglial vulnerability of the immature white matter result in significant white matter injury and permanent neurodevelopmental impairment and disability [3]. Periventricular leukomalacia is one important correlate of the neurologic injury with significant morbidity in infants born at less than 1500 g. Approximately 10% of these infants exhibit cerebral palsy and 50% exhibit cognitive and behavioral deficits [4].

Cytokines and chemokines are known to be primary modulators of white matter injury in the brain of the newborn. Multiple cell types in the brain, such as microglia, astrocytes and neurons, as well as migratory cells of the innate and adaptive immune system, participate in injury via the secretion of pro-inflammatory cytokines and chemokines. Microglial cells, which also function as antigen presenting cells in the CNS, are triggered to release IL-1, IL-6 and IL-18, as well as neurotoxic substances, including excitatory amino acids [5]. Astrocytes release transforming growth factor (TGF)-β, IL-6 and the chemokines CCL3 and CCL5 [5], whereas glial cells directly enhance neuronal injury [4]. These cells also express receptors for inflammatory cytokines and chemokines allowing autocrine up-regulation of inflammatory responses to these mediators.

Increases in pro-inflammatory cytokines and chemokines in the CNS also function to recruit inflammatory cells, including granulocytes and lymphocytes, which are not normal inhabitants of the CNS. Recruitment occurs via cytokine-mediated activation of cell-type specific adhesion receptors on vascular endothelium and through the secretion of chemokines. Chemokines, such as CCL2 facilitate the accumulation of monocytes, which upon entry into the CNS, differentiate into macrophages at sites of injury. CXCL8 attracts neutrophils and CCL28, is a chemo-attractant for T- and

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B-cells. Moreover, inflammatory mediators and cells of the innate and adaptive immune system also access the CNS more freely across a disrupted blood brain barrier (BBB) thereby minimizing the requirement for receptor-mediated migration of effector cells and soluble mediators.

Direct evidence that pro-inflammatory cytokines and chemokines damage the developing brain comes mainly from experimental studies in animal models where the type of injury and its duration can be controlled. Injection of IL-1 leads to neuronal death and delayed myelination in neonatal rats [6]. Blocking of chemokines or their receptors inhibits neutrophil-mediated damage to the BBB in experimental models. In addition, chemokine receptor inhibitors prevent CXCL8 responses and reverse the lethal sequelae of sepsis in mice [4]. Finally, TNF-α induces cell death in mature human oligodendrocytes and, in the developing ones, is associated with increased apoptosis and reduced myelination [6]. Today, there is increasing evidence that inherited cytokine or chemokine polymorphisms influence the risk for pre- and perinatal brain damage [6].

110.3 Inflammation and Injury Caused by Hypoxia-Ischemia

Birth asphyxia is an important cause of H-I injury, perinatal mortality and lifelong neurodevelopmental morbidity, including cerebral palsy, learning disabilities and mental retardation in the newborn. Perinatal asphyxia occurs in approximately 4/1000 term births and is more frequent in preterm newborns [7]. During H-I injury, glutamate excitotoxicity, free radical and pro-inflammatory cytokine release results in injury to the brain [4]. The inflammatory response to tissue injury occurs in response to the excitotoxic cascade during the reperfusion period and contributes to the evolution of injury. Free radicals produced by activated inflammatory cells activate NF-κB in brain cells leading to pro-inflammatory cytokine and chemokine release [4]. Furthermore, according to the "danger model" theory, signals from stressed or damaged cells in the CNS may initiate an immune response during which inflammatory cytokines and chemokines are also produced further contributing to injury [4].

Pro-inflammatory cytokine blood levels of IL-6, IL-1β, IL-12 and TNF-α are increased in infants with H-I encephalopathy and are associated with abnormal neurodevelopmental outcomes [4]. We have found that IL-6 and IL-1β serum levels are increased compared to controls, but these levels do not differ between asphyxiated and infected neonates [4]. However, TNF-α levels are similar between infants with H-I insults and controls. Furthermore, cytokine increases correlate with the severity of the perinatal insults [4]. In support, Okazaki et al demonstrated that serum IL-6, CXCL8 and IL-10 levels in asphyxiated terms are higher than those of the controls, while IFN-γ is lower [8]. Premature infants with MRI-defined cerebral white matter injury have higher levels of IL-6, IL-10 and TNF-α in the cerebrospinal fluid (CSF) than in the serum. There is no correlation between CSF and serum cytokine levels [6]. Finally, increased levels of activin-A, a cytokine hardly studied in term newborns, are observed in premature infants with perinatal hypoxia [9].

Elevated serum chemokines are also found in response to H-I injury. CXCL8, known to be chemotactic for neutrophils, basophils and lymphocytes, is elevated in term newborns with abnormalities on MRI and adverse neurologic outcome following H-I injury [4]. We have found increased levels of CXCL8 and CXCL10 in the blood of neonates with perinatal asphyxia during the first 24 hrs of life [4]. Our studies have also demonstrated a dichotomy of reactivity of activated peripheral blood lymphocytes according to the inflammatory insult; during perinatal infection these cells express increased levels of CXCL8 mRNA whereas during asphyxia, they express increased levels of CCL2 mRNA [10].

110.4 Inflammation and Injury Caused by Perinatal Infection

Perinatal infections are known to play an important role in the pathogenesis of white matter injury in the brain as well as in the pathogenesis of preterm labor and the later development of chronic lung disease. In fact, intrauterine infection and inflammation complicate up to 35% of preterm deliveries compared with 10–15% of those infants delivering at term [11]. Early onset sepsis occurs in 15–19/1000 live births in infants born as less than 1500 g, however, the overall risk of infection among preterm infants is greatest after the first week of life [11].

The primary barrier to infection in the fetus is the uterine-placental barrier, which has innate immune properties, in addition to innate and adaptive immune responses provided by circulating cells of the maternal immune system. In addition, passive immunity from the active transport of immunoglobulin G (IgG) across the placenta imparts additional protection to infants born at term although coverage is less complete in the premature infant as transfer is incomplete until the last trimester. The innate immune functions of uterine and placental tissues derive from the expression of toll-like receptors (TLR) [12]. TLR are pattern recognition receptors (PRR) that initiate inflammatory responses after binding a variety of viral nucleic acids and bacterial products [13]. TLR signaling further elicits anti-microbial and inflammatory responses through production of free radicals, proteases and inflammatory mediators. Ureaplasma species are implicated in inflammation induced at the feto-maternal interface [14]. Chorioamnionitis is associated with increased levels of CXCL8 and IL-6, while microbial invasion of amniotic fluid is associated with increased levels of IL-6, CXCL8 and IL-18 [14]. The fetal inflammatory response syndrome correlates

with elevated cord blood IL-6 [14]. More than 30 years ago, Dammann and Leviton showed that a strong inflammatory challenge, such as intrauterine infection, elicits a fetal inflammatory response and contributes to brain damage in preterm infants. Since then, studies have revealed that inflammatory mediators, such as cytokines and chemokines, are important links between infection and brain damage [15].

All aspects of innate and adaptive immune function after birth are immature and are further compromised in the prematurely born infant. Innate immune responses are initiated via the activation of complement, the release of anti-microbial peptides by neutrophils, paneth cells and macrophages and the engulfment of bacteria by phagocytes. Activation of the adaptive immune system occurs following antigen processing and presentation by antigen presenting cells, such as dendritic cells. Complement proteins, Ig and anti-microbial peptides promote direct cell killing following the release of anti-microbial peptides and enzymes, as well as phagocytosis through opsonization by Ig. Paneth cells, at the base of the crypts in the intestine and in other mucosal sites produce a variety of antibacterial substances including defensins and cathelicidins [16, 17]. These are small cationic peptides with broad-spectrum antimicrobial action, released in response to bacteria or to components of bacterial cell walls [18]. They are found at significantly lower levels in the intestine during fetal life when compared with the term newborn and adult [19]. Thus, the decreased production of defensins by paneth cells may predispose the fetus or infants born prematurely to bacterial overgrowth and overwhelming infection.

The production of granulocytes in the bone marrow and their progenitors is limited in the term and premature infant and their function is impaired potentially contributing to bacterial overgrowth and invasion. Likewise, the inflammatory cascade induced by bacteria may include a reduced oxidative burst in the premature infant due to low levels of NADPH, thereby reducing the function of the innate immune system [20]. Regarding adaptive immunity, although considerable development of T- and B-cells occurs during fetal life, complete maturation of the immune system occurs after birth. The production of antibodies by B-cells may be delayed and lower levels of Ig are detected despite the active transport of maternal IgG. Moreover, antigen uptake, degradation, and presentation by antigen presenting cells, a vital step in the initiation of an adaptive immune response, is less efficient in the newborn, hence, the ability of the adaptive immune system to detect and respond to pathogenic organisms is impaired. T-cells in the newborn are also less responsive to antigenic stimulation and have a decreased proliferative response to a variety of mitogenic stimuli [21]. Finally, circulating and epithelial-associated levels of IgA and IgG are also reduced resulting in greater susceptibility of the newborn to infections and across mucosal surfaces [22].

Regarding cytokines and chemokines in neonatal inflammation, increased levels of IL-6, CXCL8 and TNF-α are observed in newborns with early-onset sepsis [6]. Elevated levels of IL-6, IL-12p70, IL-10 and TNF-α levels are higher in LBW infants compared to controls, but these levels decrease after 24 hours with late-onset sepsis [23]. Premature newborns produce increased levels of pro-inflammatory cytokines, such as IL-2, IL-6, IFN-γ, TNF-α, and anti-inflammatory cytokines, such as IL-4 and IL-10 during infection compared to non-infected controls [23]. Yerkovich et al recently showed that IL-6, IL-10, TNF-α and IFN-γ release, following *in vitro* lipopolysaccharide (LPS) stimulation of cord blood cells, is equal or greater than that of adults [24]. The chemokine CXCL8 has been extensively studied and considered as a serum marker of neonatal sepsis, particularly in the early-phases of disease [4]. CXCL8, CXCL10, CCL9, CCL2, CXCL1 are all increased in neonates with sepsis, but their levels decrease after 24 hours whereas CCL5 is similar to non-infected controls [23]. Interestingly, Tatad et al proposed that the type of infectious agent leads either to increased inflammation or defective immunity. For example a lower level of IL-6 in response to *Staphylococcus epidermidis* infection may indicate a basis for vulnerability to infection whereas a high CXCL8 response to pathogenic *Escherichia coli* in preterm newborns is consistent with uncontrolled inflammation [25]. Taken together these results suggest that it is not only the absolute level of cytokines, but also the balance for pro-inflammatory cytokines and their counter-regulatory anti-inflammatory counterparts that may be important in disease pathogenesis.

The pathophysiology of an infectious disease in the fetus and newborn may be exacerbated by the inflammatory response to the pathogen rather than by virulence characteristics of the pathogen. In fact, excessive secretion of pro-inflammatory cytokines and chemokines may be more damaging to the host than the pathogen itself. Certain studies indicate that neonates have an increased inflammatory response compared to adults [24, 26]. Our findings also showed that LPS-induced TLR4, CD14 expression and CXCL8 release by neonatal peripheral blood leukocytes were significantly increased compared to adults [27]. Thus, quite often an overwhelming systemic inflammatory response may be generated during early or late -onset sepsis, meningitis or necrotizing enterocolitis, resulting in multi-organ failure, brain injury or death. Infants with infections are more likely to have brain damage [11].

Moreover, as the susceptibility of the newborn infant to infection is well-known, it is not unreasonable to propose that multiple defects in both innate and adaptive immunity contribute to further dysregulation of cytokine and chemokine secretion and account for significant "bystander injury" in organs such as the brain.

It is also important to note that physiologic responses to overwhelming bacteremia and sepsis may lead to microcirculatory dysfunction, shock and cerebral hypo-perfusion, resulting in pathways that overlap with primary H-I injury in the brain (Fig. 110.1). Hence, determining the primary root

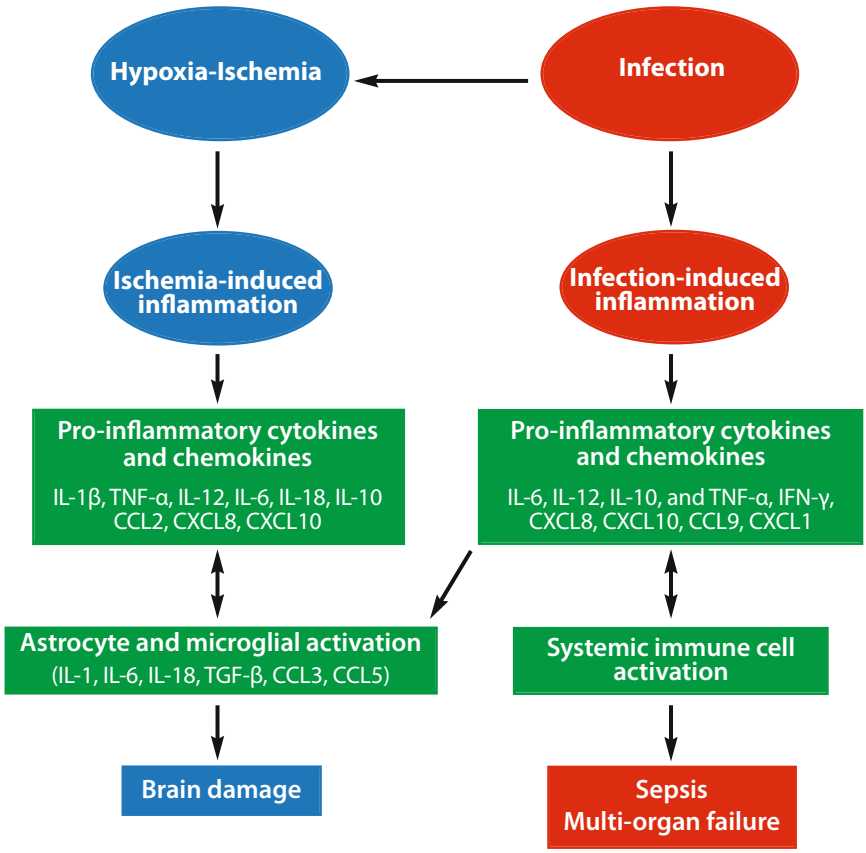

Fig. 110.1 H-I insults and infection lead to inflammation mediated by pro-inflammatory cytokines and chemokines produced by cells resident in the brain as well as by migratory bone marrow-derived immune cells. These mediators may either enter the brain and lead to white matter injury or cause systemic immune cell activation resulting in sepsis and multi-organ failure. Overwhelming infection can lead to H-I

of cause and effect of white matter injury in the brain during sepsis and shock may be difficult.

110.5 Cytokines and Chemokines as Therapeutic Targets in H-I Injury and Infection

Levels and ratios of pro-inflammatory cytokines and chemokines may provide the clinician with valuable diagnostic and prognostic information regarding inflammation induced by asphyxia and/or infection; however validation of these markers as prognostic indicators or as targets for treatment still needs to be documented. As yet, no definitive pattern or level of biomarker expression has been found to be predictive of disease severity or outcome, although certain cytokines and chemokines have been proposed [28].

Therapeutic interventions that either enhance the production or activity of anti-inflammatory cytokines or inhibit the production or activity of pro-inflammatory cytokines have been used quite successfully in a variety of animal disease models and in management of autoimmune and inflammatory diseases in humans such as in rheumatoid arthritis [29] and in inflammatory bowel disease [30]. However, clinical studies directed at modulating cytokine or chemokine function in newborn infants following H-I injury or infection with the goal of preventing overwhelming sepsis and minimizing white matter injury in the brain along with improving long-term outcome, are promising areas of research. Overall, targeting specific combinations of cytokines, chemokines and/or their receptors on brain and immune cells may inhibit excessive inflammation and injury, while sparing beneficial responses and protective immunity to infections.

110.6 Conclusions

Although neonates exhibit impaired expression of certain inflammatory cytokines and chemokines, such as IFN-γ, IL-12 and CCL5, the majority of inflammatory mediators are increased during infection and asphyxia to adult or more than adult levels. This could reflect the excessive inflammation often observed in neonates and, more so in premature infants, which can lead to septic shock and brain damage or the inability to mount an appropriate counter-regulatory anti-inflammatory response.

Understanding the mechanisms that contribute to the production of these pro-inflammatory cytokines and chemokines may lead to the identification of important therapeutic targets for brain damage and sepsis.

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111

Neonatal Malignancies

Franca Fossati-Bellani

111.1 Introduction

Neoplastic or tumor-like conditions are rare in the fetus and newborn, but pose important diagnostic and therapeutic problems for pediatricians and oncologists, as well as imposing enormous emotional burdens on parents and caregivers. They also present significant medical and ethical dilemmas.

By definition, neonatal neoplasms are those diagnosed during the first month of life. Congenital neoplasms are those diagnosed at birth. It is essential to define and distinguish tumor-like conditions (such as hamartomas, hemangiomas, lymphangiomas and melanocytic nevi) from benign or malignant tumors. Tumors that are benign at histology can still grow aggressively, acquiring a malignant behavior due to their site of onset and local invasiveness. They can cause death, as in the case of extensive lymphangiomas and teratomas of the newborn in the head, neck or mediastinum. On the other hand, histologically malignant tumors of the newborn, such as neuroblastoma stage 4S and neonatal fibrosarcoma, can regress spontaneously and take a benign course even without therapy. Finally, there are non-neoplastic conditions (i.e., abdominal masses) that can simulate the presence of a tumor, and that have to be differentiated by appropriate diagnostic procedures (Table 111.1). The vast majority of neonatal neoplasms are solid tumors of mesenchymal and embryonic origin, and they can be diagnosed before birth. Because they are so rare, the management of these tumors represents a major challenge for neonatologists, surgeons and oncologists: the knowledge gained from infants cannot be applied to the newborn because of the physiological immaturity of the latter's metabolism, hemopoietic and immune systems. Furthermore, current therapeutic protocols used in cooperative studies on childhood tumors fail to consider the peculiarities of the newborn.

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111.1.1 Incidence

Data on the incidence of neonatal malignancies are drawn from single-institution case studies or tumor registries, but there are no reliable general population based data available. Their incidence is estimated to be approximately 1 in every 12,500–27,500 live births. Neonatal malignancies account for 2% of all tumors affecting infants, children and adolescents. Half of neonatal tumors are found at birth, 20–30% during the first week and the remaining 20–30% within the first month of life. During the neonatal period, the incidence of a given tumor does not coincide with the related mortality rate because some tumors are rapidly lethal, while others cause death after the neonatal period, and yet others grow initially and then regress spontaneously, e.g., cystic neuroblastoma (NBL) and NBL in stage 4S. NBLs and teratomas (75% benign and 25% malignant) are the most frequent neonatal neoplastic conditions (accounting for 22% and 23%, respectively), followed by soft tissue sarcomas (8%), acute leukemias (6%), central nervous system (CNS) tumors (6%), benign and malignant liver tumors (6%), kidney tumors (7%), and retinoblastomas (5%). Males and females are generally affected equally, but retinoblastoma is more common in males, teratoma in females. Leukemias and CNS tumors are the most lethal in this age group.

111.1.2 Etiology

Neonatal congenital tumors constitute a unique model for studying the oncogenetics. The aging process and multiple environmental factors considered in adult studies are not applicable to the etiopathogenesis of tumors in children. Chemical and physical factors, and infections can influence the oncogenic processes involving the gametes, embryo and fetus. Genetic factors involved in the development of neoplasms, such as small mutations, the loss of heterozygosis and

G. Buonocore et al. (eds.), *Neonatology. A Practical Approach to Neonatal Diseases*.

Table 111.1 Malignant (a), non malignant (b), tumor like conditions (c) of the newborn by location

Brain	a. Teratoma, PNET (medulloblastoma, pinealoblastoma, cerebral neuroblastoma), choroid plexus carcinoma, ependymoma, astrocytoma, atipical teratoid rhabdoid tumor, sarcoma, melanoma b. Teratoma*, meningioma*, hamartoma*, craniopharyngioma* c. Vascular malformation, hemorrage, hydrocephalus
Head and neck	a. Rhabdomyosarcoma (orbit, nasopharynx, middle ear), neuroblastoma (neck), retinoblastoma, teratoma, melanotic progonoma, Langherans cell histiocytosis b. Teratoma*, fibromatosis*, melanotic progonoma*, hamartoma, brachial cyst, lymphangioma* c. Cellulitis, nasopharyngeal brain heterotopia
Trunk	a. Neuroblastoma, germ cell, soft tissue sarcoma, Langherans cell histiocytosis b. Lymphangioma*, hamartoma*, teratoma*, cardiac rhabdomyoma c. Pulmonary myofibroblastic tumor, massive mesenchymal malformation of lung
Abdomen pelvis	a. Neuroblastoma, renal tumors, hepatoblastoma, germ cell tumors, soft tissue sarcoma, acute leukemia b. Teratoma, hepatic hamartoma, hemangioma, hemangioendothelioma, congenital mesoblastic nephroma* c. Polycystic kidney, hydronephrosis, urinary retention, gastrointestinal duplication, storage disease ovarian cyst, congenital viral infections
Skin and superficial soft tissue	a. Soft tissue sarcoma, neuroblastoma, acute leukemia, Langherans cell histiocytosis, melanoma b. Hemangioma, fibromatosis, giant naevus c. Infections

* Benign tumors potentially life threatening because of size and location or tendency towards malignant transformation.

changes in genomic imprinting, are more prominent in children than in adults. The correlation between malformations, genetic syndromes (hemihypertrophy, aniridia, Beckwith-Wiedemann syndrome) and tumors of childhood points to the conclusion that genetic factors are more significant than environmental ones in neonatal neoplasms.

111.1.3 Clinical Features and Diagnosis

Most tumors are clinically manifest as an abnormal mass located in the abdomen or head and neck, or any soft tissue site. They may be detected before birth. Advances in diagnostic procedures, e.g., ultrasound (US) and magnetic resonance imaging (MR), have facilitated the prenatal diagnosis of congenital neoplasms such as teratomas, abdominal or intrathoracic masses, with consequent implications for prenatal therapy and the choice of vaginal versus cesarean deliveries, and for the outcome of the fetus.

MR provides details of the site and the anatomical extent of a lesion and, in selected cases, it has been instrumental in strategic prenatal therapeutic procedures, such as fetal surgery or fetal rescue at the time of delivery. Computerized tomography (CT) is a diagnostic tool commonly used to diagnose tumors postnatally, but MR performs better in evaluating CNS neoplasms and spinal cord compression. Positron emission tomography (PET) has yet to be validated for use in diagnosing childhood tumors. Imaging data, combined with clinical findings, provide the basis for the histopathological diagnosis of neoplastic lesions that have been totally resected or biopsied. To characterize a neoplasm precisely, the morphological features derived from classic histology must be combined with immunohistochemistry and cytogenetic assessment. For instance, the value of the N-myc oncogene (a molecular marker of NBL) is significant in terms of prognosis and treatment.

111.1.4 Principles of Therapy

Interdisciplinary cooperation between all specialists involved at the various stages of the diagnostic and therapeutic process is vital.

111.1.4.1 Surgery

Surgical ablation is the therapeutic procedure of choice for most solid tumors (such as teratomas, Wilms' tumor and neuroblastoma).

Pediatric surgeons do not have to undertake aggressive surgery. The timing and strategy of surgical procedures have to consider the newborn's metabolic and physiologic needs, as well as the local extent of the tumor to enable complete excision, and the feasibility of delaying surgery until after shrinking the tumor volume by chemotherapy to avoid mutilating surgery or operations that would interfere with the child's growth or impair any vital functions. In pediatric oncology, the purpose of a multidisciplinary approach is to limit the damage associated with invasive therapies, especially in younger patients, without affecting adversely survival prospects.

111.1.4.2 Chemotherapy (CHT)

There are not enough pharmacologic studies of drug metabolism, clearance and toxicity to organs and functions to support the use of antitumor drugs in the newborn. Data on the pharmacology of antitumor chemotherapy in infants and young children have revealed severe neurotoxicity of vincristine, hepatotoxicity of actinomycin D, myelotoxicity of adriamycin and ototoxicity of cisplatin. Traditional cytotoxic drugs, administered at doses adjusted to the infant's weight enable drug clearance from the patient with fewer global toxic effects and a good therapeutic index. Using a patient's weight to guide dosage has been empirically adopted for the newborn. Drug dosage must be modified in the event of major side effects.

111.1.4.3 Radiation Therapy

The adverse effects of radiation therapy on infants and young children are now well known. They include irreversible damage to growth, the musculoskeletal system, and endocrine and cognitive functions, depending on the site and volume of the radiation fields. Furthermore, the risk of second tumors limits the use of RT in pediatric oncology and makes it contraindicated for the newborn.

111.1.4.4 Supportive Care

In pediatric oncology, good therapeutic results have been achieved (now reaching a 5-year survival rate of 70%) because of improved supportive care. Hydration, antibiotics, blood products, growth factors, antiemetic agents, and nutritional support are all particularly important for the newborn because of their inability to withstand invasive treatments and because of the severe risk of infection and metabolic complications in the short term. Central venous access is important [1–6].

111.2 Tumors

111.2.1 Neuroblastoma

NBL originates from neural crest cells and can occur along the sympathetic chain and in the adrenal medulla. The link identified at autopsy between infants dying of other causes and a high incidence of NBL in situ might be explained by this tumor's tendency for spontaneous regression. Most NBLs are associated with chromosomal anomalies, some of the most frequent being amplification of the oncogene N-myc, deletion of chromosome 1p, and aneuploidy. An amplified N-myc carries a poor prognosis [5-9].

111.2.1.1 Clinical Findings

The most common finding is an abdominal mass originating from the adrenal gland, but the primary tumor may be located in the mediastinum and neck, retroperitoneum or pelvis. Abdominal distension with or without respiratory insufficiency is a clinical finding that correlates with massive liver involvement from a small adrenal tumor. Secondary subcutaneous nodules and bone marrow invasion are also observed in this stage, defined as 4S. Other symptoms are due to the mass effect of the tumor, according to its location (respiratory insufficiency, or Horner syndrome if it is in the neck or mediastinal region) and/or to any metastasis.

111.2.1.2 Prenatal Diagnosis

When prenatal US identifies an abdominal mass, MR can differentiate between NBL and other tumors. In the prenatal phase, adrenal cystic lesions are interpreted as persistent or late-regressing neuroblastic nodules. They tend to regress spontaneously and they do not alter catecholamine metabolite levels, which can cause hypertension or maternal pre-eclampsia when abnormal.

111.2.1.3 Postnatal Diagnosis

Both CT and MR can distinguish between adrenal hemorrhages, renal masses and intra-abdominal sequestration (Figs. 111.1 and 111.2). Methyl iodobenzylguanidine (MIBG) scintigraphy can be used both to identify a neuroblastic tumor and to detect metastases. Only 70% of neonatal NBLs are MIBG-avid.

Histology is the final diagnostic tool, together with the previously-mentioned prognostic markers (N-myc, 1p deletion). If the liver is extensively affected (stage 4S), liver needle biopsy has been used instead of open abdominal surgery to reduce the risk of respiratory impairment and complications related to abdominal healing.

111.2.1.4 Staging

The INSS (Intergroup Neuroblastoma Staging System) criteria are used.

111.2.1.5 Therapy and Prognosis

The treatment program depends on the stage of the tumor and its biological characteristics. Children under one year or infants have a significantly better prognosis than older children. Currently cooperative groups recommend monitoring cystic

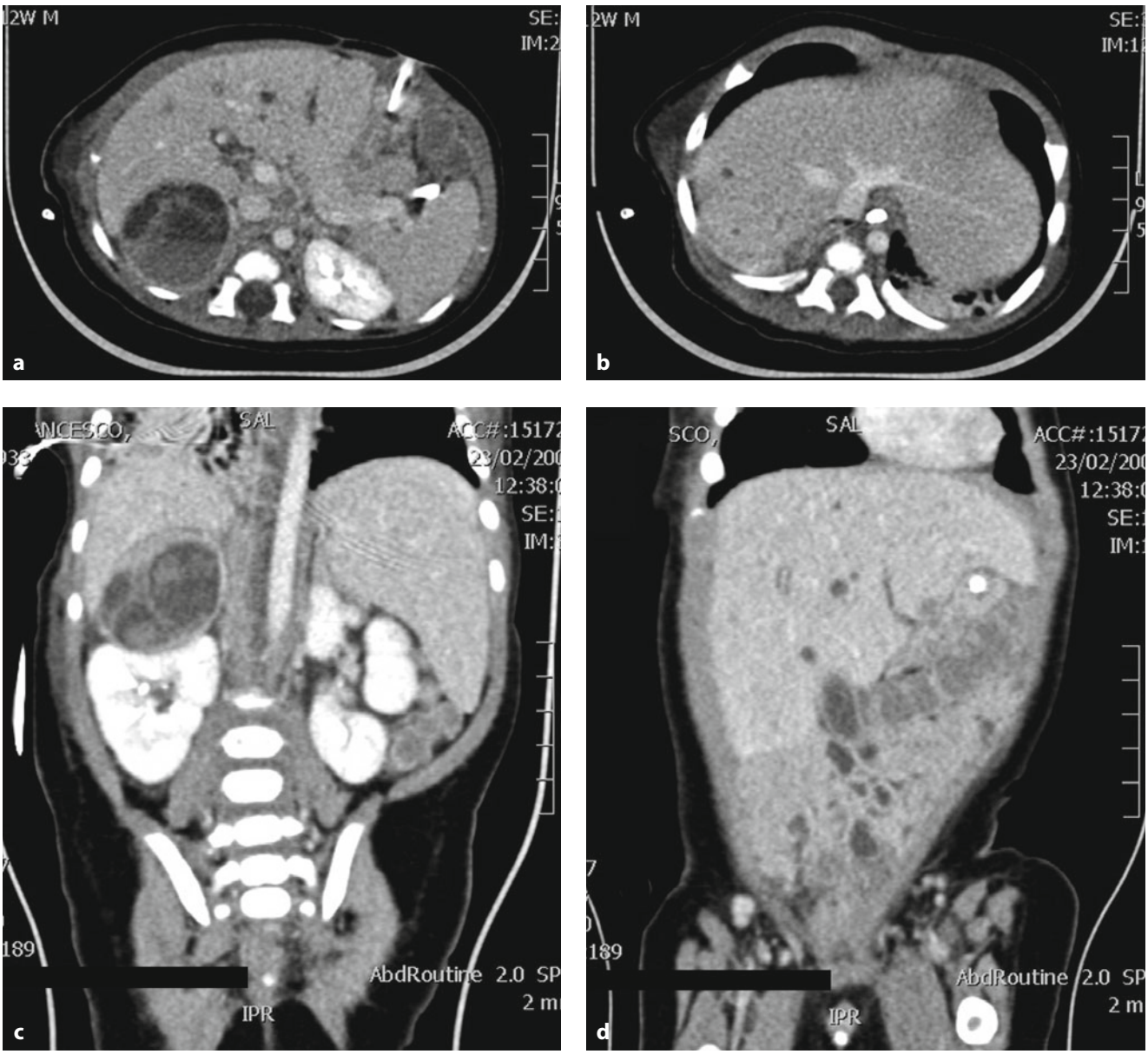

Fig. 111.1 Neuroblastoma 4S CT imaging. Right adrenal lesion, mostly cystic necrotic (**a**–**c**) with hematic level. Multiple metastatic little epatic lesions are present (**b**–**d**). (Courtesy Dr. Marcello Napolitano)

NBL during the first three months by US. Surgical resection is recommended when it neither regresses nor increases in size. Newborns in stages 1 and 2 have a good prognosis with surgery alone even when histology shows microscopic residues but no N-myc amplification in stage 2 cases. NBLs classified as stages 3 and 4 are prevalent in children over one year. Chemotherapy (CHT) is performed first to shrink the tumor, delaying surgery until later. For stage 3, the prognosis is excellent (about 90%), while for stage 4 it is 50%. If N-myc is amplified, high-dose intensive CHT with bone marrow rescue is recommended.

The prognosis is poor, however, especially when more than 10 pairs of the N-myc oncogene are identified. The drugs used are carboplatin, adriamycin, vincristine and topotecan. Newborns with stage 4S NBL have a good prognosis in 80% of cases without specific therapy. In cases with hepatic involvement and a risk of respiratory deficiency and/or vena cava compression, CHT can be implemented to accelerate tumor regression; surgery for the primary tumor is considered unnecessary. CHT and surgery are essential, however, in cases with biological markers indicating a poor prognosis [5–9].

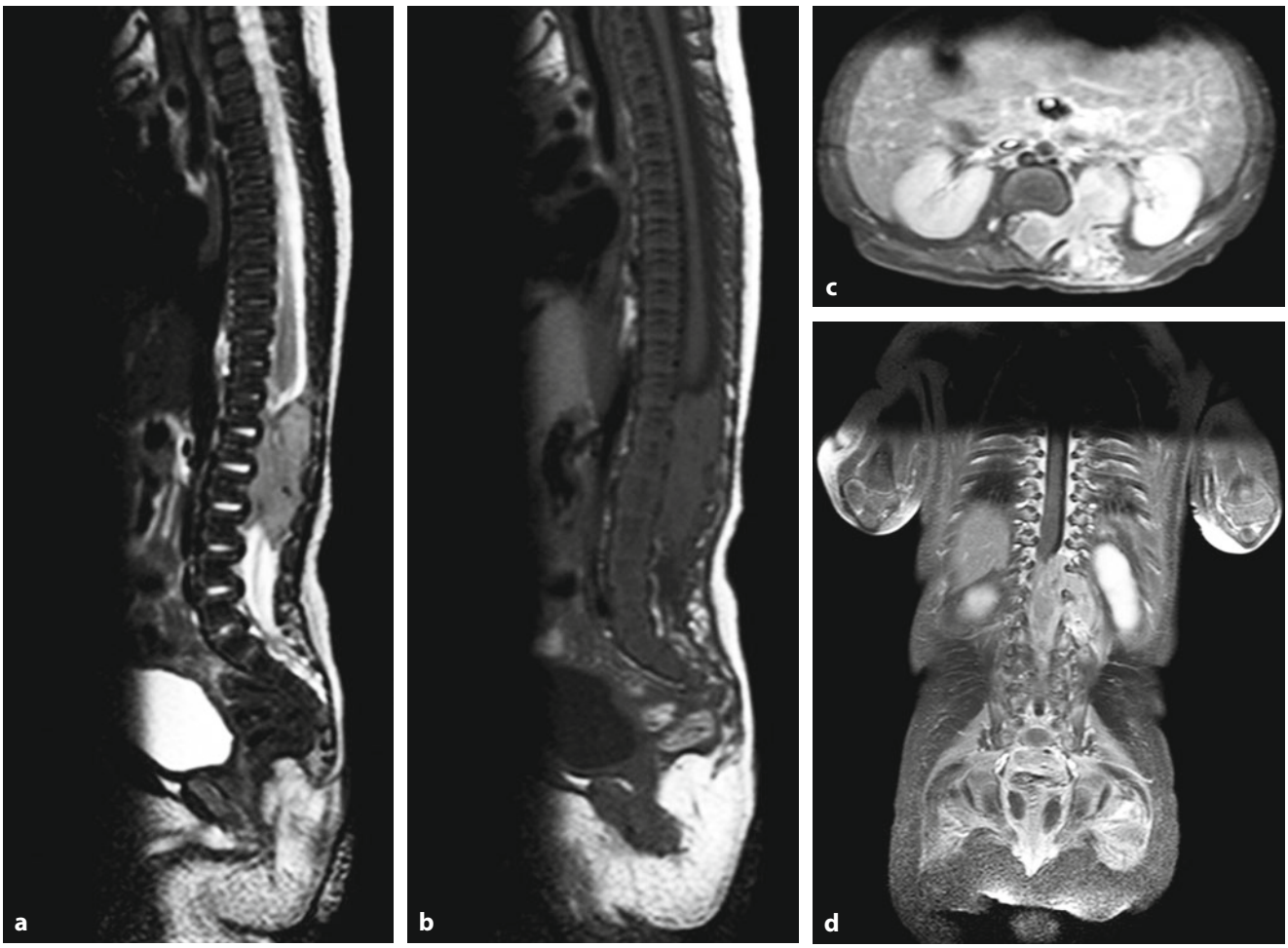

Fig. 111.2 Paravertebral left neuroblastoma with paraspinal and intrarachideal infiltration through conjugation foramina. MR imaging T2 W (**a**) and T1 W pre (**b**) and after (**c**, **d**) gadolinium. (Courtesy Dr. Cecilia Parazzini)

111.2.2 Germinal Tumors (GT) and Teratomas (TRT)

These are the most frequent benign and malignant perinatal tumors. More common in females, they are embryonic tumors that originate from primordial germ cells or germ layers (the ectoderm, mesoderm and endoderm). The World Health Organization (WHO) classification divides GT into 7 categories, but only TRT, yolk sac tumor and, more rarely, gonadoblastoma have been described in the perinatal period. More than 50% of TRTs appear at birth in the sacrococcygeal region, but they can occasionally be found in the head and neck, mediastinum, brain, retroperitoneum, and liver. TRTs are classified into three different groups, depending on their histology: (a) mature, consisting mainly of adult tissue; (b) immature, consisting of embryonic tissue; or (c) malignant, consisting of malignant embryonic tissue, which is almost always a yolk sac tumor.

The germinal tumor marker alpha-fetoprotein (AFP) levels are significantly abnormal in the malignant form and must be measured and monitored over time (considering as normal the levels during its normal regression in the first year of life). Approximately 10% of neonatal TRTs contain malignant cells, while 20% have immature components. Neonatal TRTs are often found in association with multiple or combined malformations of the genitourinary tract, rectum, anus and sacrococcygeal region, or of the face in the head and neck. Sacrococcygeal TRT may be post-sacral (external), pre-sacral (internal), or both (dumbbell). They are solid, cystic, or mixed masses containing varying proportions of all tissues, i.e., fat, muscle, bone, teeth, hair, immature neuroepithelial glandular tissue and glia. Prenatal assessment of the volume and site of the tumor is used to manage the delivery and to decide on perinatal measures such as intubation for airway obstruction in the case of cervical and mediastinal involvement. Treatment is surgical: open fetal surgery is indicated for large sacrococcygeal tumors when cardiovascular complications

and hydrops are present in mother. After delivery, the sacrum and coccyx may be removed. There must be accurate histopathology to evaluate the quality of the surgical excision, the entity and characteristics of the various cytological components, and the presence of immature or malignant tissue. Local relapses may occur in 10% of mature/immature tumors, especially if the surgical resection was marginal, or if the coccyx was not removed. The introduction of CHT with cisplatin plus etoposide in metastatic disease or for initially inoperable tumors has achieved complete regression of metastatic deposits and enabled radical surgery by reducing the tumor's initial volume. This strategy allowed a spectacular improvement in survival in both locally advanced and metastatic disease (the prognosis is good in 90% of cases of localized disease and 80% of metastatic cases). Adverse prognostic factors are even minimal presence of yolk sac tumor cells in mature and immature TRT and an incomplete resection [5, 6, 10–12].

111.2.3 Soft Tissue Sarcoma (STS)

Benign and malignant soft tissue tumors are the neoplasms most commonly seen in newborns after teratomas. They include conditions that differ in histology, molecular characteristics, biological behavior and clinical evolution. They also differ from the STS seen in adults. Malignancies include rhabdomyosarcoma (RMS), congenital fibrosarcoma, and adult-type sarcomas. These have to be differentiated from benign tumors and tumor-like conditions such as vascular lesions and fibromatosis [5, 6, 13, 14].

111.2.3.1 Rhabdomyosarcoma (RMS)

This is the most common malignant sarcoma to be found in the newborn, but only 14 cases out of 3217 (4%) were reported in the study of the American IRS (Intergroup Rhabdomyosarcoma Study). The embryonal histotype is most common, while the alveolar one is rare. RMS can occur anywhere in the body, e.g. in the head, neck, genitourinary area, and limbs. Diagnostic procedures include CT, MR, bone marrow aspiration and radiographs of the skeleton. Metastases may already be present at diagnosis. Symptoms may vary, e.g., acute urine retention (when the RMS is located in the pelvis) or cranial nerve palsy (when it affects the head-neck region), due to swelling in the area affected by the tumor. The botryoid variety includes grape-like neoformations, especially in the vulvovaginal area or mouth. Treatment depends on the stage of the tumor and is the same as for children under one year (or infants), as established in cooperative international studies. A combination of CHT and surgery is used, depending on the location and extent of the tumor, and whether it is amenable to surgical resection. Surgery may be the first step for small lesions, since it may be both diagnostic and therapeutic. Where surgery fails to completely remove the tumor, a second-look surgery is recommended after CHT. A combination of vincristine, actinomycin D and cyclophosphamide is the first therapeutic choice for inoperable or metastatic disease. The prognosis is less favorable in the newborn than in older children, especially in cases with the alveolar histotype.

111.2.3.2 Congenital Fibrosarcoma

This tumor occurs in the newborn and up to six months of age. It mainly affects the limbs, but the trunk, the sacrococcygeal and retroperitoneal regions, and the head and neck may also be involved (Fig. 111.3). The characteristic chromosomal translocation t(12:15) involving genes ETV6 and NTRK3 in the newborn does not appear in older children. For cases of locally extensive disease, primary CHT can shrink the tumor and delay the need for surgery.

111.2.3.3 Other Soft Tissue Sarcomas

The atypical teratoid rhabdoid tumor (ATRT) of soft tissues has the same chromosomal abnormalities, or INI gene mutations, as those observed in the more common ATRTs of the kidney, liver and brain of children more than one year old. It is rare in soft tissues and carries a poor prognosis. There are reports in the literature of other types of STS in the newborn, including peripheral primitive neuroectodermal tumor (pPNET), vascular tumor and undifferentiated sarcomas.

111.2.3.4 Congenital Fibromatosis

This is a tumor-like condition that needs to be distinguished from the above-mentioned malignant mesenchymal tumors. A common feature of congenital fibromatosis is the proliferation of elongated fibroblastic cells that tend to invade the surrounding tissues, but do not give rise to metastases.

a. Solitary or multicentric congenital myofibromatosis is more common in males and affects the head and neck, trunk and skin. It may regress spontaneously.
b. A sternomastoid tumor is generally connected with birth trauma. It must be differentiated from cervical NBL and RMSA.
c. Infant desmoid-type fibromatosis is found in muscles, aponeuroses, shoulders, the head and neck, and the upper limbs.
d. Cranial fasciitis can grow rapidly and infiltrate the skull bone.
e. Fibrous hamartoma of infancy (which is congenital in 20% of cases) develops in the cutaneous tissue of the shoulders, arms, thighs, armpits and inguinal areas. It is composed of fibrous, adipose and immature mesenchymal cells, and is treated with surgery.

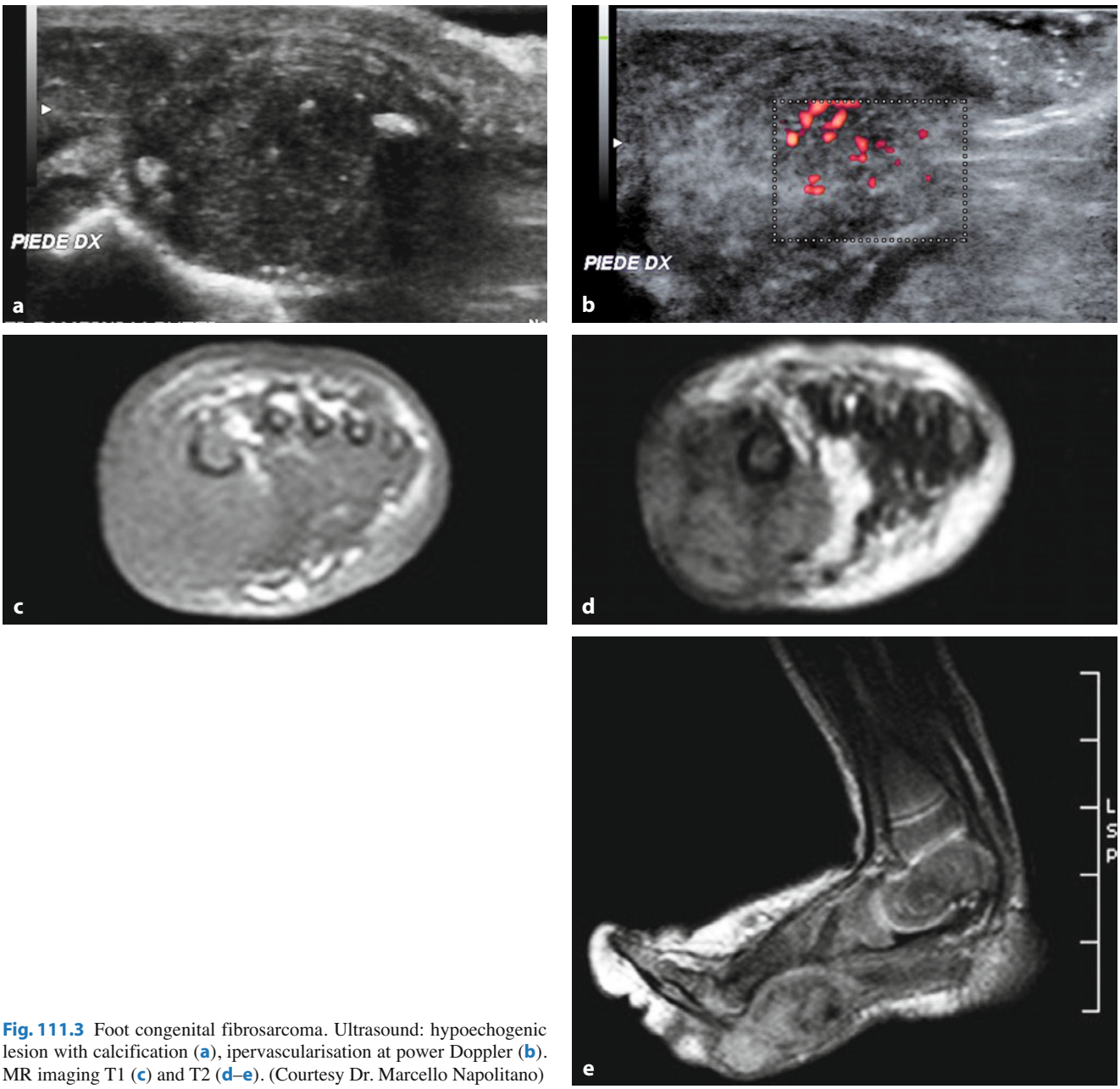

Fig. 111.3 Foot congenital fibrosarcoma. Ultrasound: hypoechogenic lesion with calcification (**a**), ipervascularisation at power Doppler (**b**). MR imaging T1 (**c**) and T2 (**d**–**e**). (Courtesy Dr. Marcello Napolitano)

111.2.4 Kidney Tumors

Neonatal kidney masses are benign in more than 40% of cases. Congenital mesoblastic nephroma, Wilms' tumor and RT are, in order of prevalence, the main perinatal renal neoplasms, but clear cell sarcoma of the kidney can also occur [5, 6, 15–17].

111.2.4.1 Congenital Mesoblastic Nephroma

These account for two thirds of all solid tumors in newborns. It is a mesenchymal tumor resembling infantile fibromatosis. It is generally benign and curable with surgery alone. It can be diagnosed before birth by US, and it has no imaging characteristics to distinguish it from Wilms' tumor, even in the postnatal phase. It is more common in males.

In addition to the abdominal mass, there may be symptoms such as hypertension, hematuria and vomiting. The tumor can grow beyond the renal capsule and invade the surrounding tissues. Local recurrences are observed if the tumor is ruptured during surgery. Distant metastases are rare, especially in the cellular type. The survival rate is better than 90%. Drugs are used for local recurrences or metastases, which however are seldom seen.

111.2.4.2 Wilms' Tumor (WT)

This occurs in nephrogenic residues persisting after the 36th week of gestation. There are no reports in the literature of WT being identified in the newborn carrying the correlated malformative syndrome, but babies with aniridia, hemihypertrophy or Beckwith-Wiedemann syndrome (organomegaly, macroglossia, hypoglycemia at birth) should be monitored to enable the tumor to be diagnosed before the onset of symptoms. The clinical sign of WT consists of an abdominal mass, which must be differentiated from other masses using US/CT/MR (Fig. 111.4). Surgery is the therapeutic procedure of choice. In addition to establishing the stage, histopathology should give information on the presence of anaplastic areas in tumor specimens, which suggest a poor prognosis.

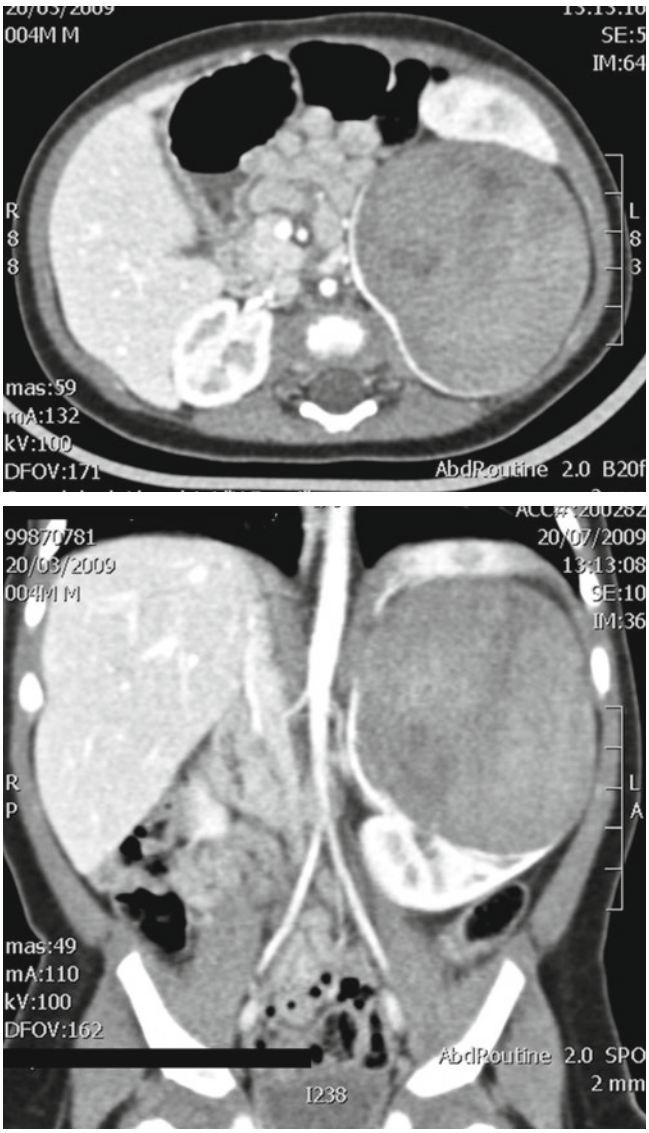

Fig. 111.4 CT imaging of Wilms' tumor with huge solid mass of left kidney

Staging methods have been based on the cooperative protocols of the COG (Children's Oncology Group) and SIOP (International Society of Pediatric Oncology). In the newborn, the tumor is generally detected in stages 1 or 2 and has a favorable histology (no anaplasia). The drugs used postoperatively include vincristine, actinomycin D and/or adriamycin, depending on the stage of disease. Ifosfamide and carboplatin are used in cases with metastases. The prognosis is extremely good (90%).

111.2.4.3 Atypical Teratoid Rhabdoid Tumor and Clear Cell Sarcoma of the Kidney

Both these diseases demand surgery whenever possible but metastases often occur. Chemotherapy has no influence on the prognosis, which is poor.

111.2.5 Brain Tumors

Congenital and neonatal CNS tumors represent 1% of all CNS tumors, and differ in terms of site, histology, clinical behavior and prognosis from those seen in older children. The most common site of onset is supratentorial. Benign cases (which are more common) may suggest malignancy due to their size, since they often cannot be removed. The most common brain tumor is a teratoma, followed by glioma, supratentorial PNET (primitive neuroectodermal tumors) (Fig. 111.5) and medulloblastoma; these last two may coincide with metastatic deposits along the spine.

All malignant CNS tumor types occurring in infants are seen in the newborn too (teratoma 50%, astrocytoma 16%, medulloblastoma 8%, choroid plexus papilloma 7%, ependymoma 3%, unclassified glioma 22%). Symptoms are unusual, macrocephaly (caused by hydrocephalus or the volume of the tumor) being the most significant clinical sign. The fontanel may pulsate. The tumor may displace normal brain without infiltrating it, which explains the absence of focal symptoms. Vomiting and papilledema are late signs, and are due to the increasing size of the neoplasm. Non-specific symptoms, such as lethargy, fever and gastrointestinal disorders, are often misinterpreted, causing delay in the correct diagnosis. MR of the brain and spine provide information on the tumor's size and its benign or malignant nature. Hemorrhagic episodes related to the neoplasm may simulate brain hemorrhage or even cause a massive brain hemorrhage. Treatment options must be discussed on a case by case basis and depend on the site and size of the lesion, imaging findings and the newborn's general conditions. Surgery may be curative in patients with benign teratoma and ependymoma. Cases of PNET and medulloblastoma require pharmacological treatment. Surgery is often only of diagnostic value. Given the poor prognosis (the survival rate is 28% in the

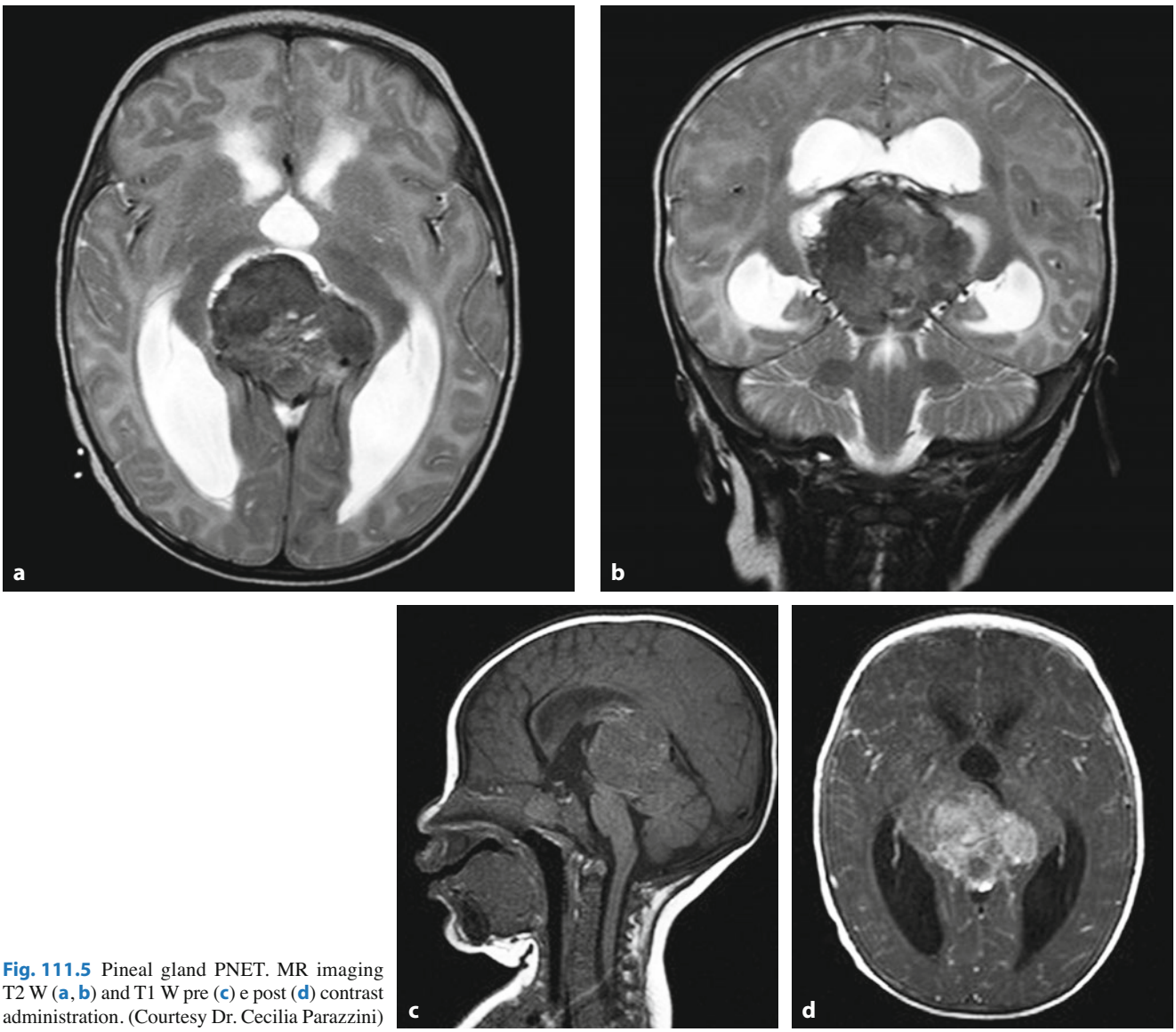

Fig. 111.5 Pineal gland PNET. MR imaging T2 W (**a**, **b**) and T1 W pre (**c**) e post (**d**) contrast administration. (Courtesy Dr. Cecilia Parazzini)

most comprehensive case studies in the literature, which include both benign and malignant lesions), non-intervention may often be the best option. An open and frank discussion between care-givers and relatives is essential before any treatment decisions are made [5, 18, 19].

111.2.6 Liver Tumors

Neonatal liver neoplasms are usually benign (hemangioma 60%, mesenchymal hamartoma 23%, hepatoblastoma 17%). Infant hemangioma is a benign vascular neoplasm, that is generally discovered incidentally during abdominal US for other reasons. There may be no clinical signs of its presence and it may not evolve, but extensive hepatic involvement may be a cause of severe and life-threatening cardiovascular complications [5, 20].

111.2.6.1 Hepatoblastoma (HB)

This is an embryonic neoplasm with variably differentiated epithelial and embryonic tissue with both embryonic and fetal components. It can be found prenatally and may be responsible for polyhydramnios and premature birth. While its association with malformative and genetic syndromes (hemihypertrophy, Beckwith-Wiedemann syndrome, familial polyposis)

is well known, hepatoblastoma has not been described during the prenatal period.

The clinical signs are abdominal distension and hepatomegaly. After imaging tests (MR/CT), the diagnosis is confirmed by a liver biopsy and abnormally high levels of alpha-fetoprotein, which represents a tumor marker. AFP is the major protein produced by fetal liver. AFP levels are markedly elevated in more than 90% of HB. One must relate the AFP values to the physiological AFP levels of premature newborns and infants. The SIOP protocol relies on primary cisplatin chemotherapy, followed by resection. Hepatoblastomas amenable to surgery have a more than 90% favorable prognosis. Liver transplantation should be considered in cases in which hepatic resection is unfeasible.

111.2.7 Leukemias

Leukemia is the most lethal disease of the newborn. It causes fetal hydrops and death, especially in Down syndrome patients. Leukemia in the newborn differs in clinical, hematological, molecular and biological aspects from the disease in older children.

Acute non-lymphoblastic leukemia (ANLL) is more common (65%) than acute lymphoblastic leukemia (ALL) and the former has a better prognosis in cases achieving remission (the survival rate is 24% for ANLL and 10% for ALL). The clinical findings include hepatosplenomegaly and skin lesions; the CNS is sometimes affected. Bone marrow cytomorphological, immunophenotypic and cytogenetic investigations are mandatory for diagnostic purposes. Congenital leukemia is CD10-negative and may be positive for markers in both the lymphoid and the myeloid lines. There is a rearrangement of the MLL (mixed linear leukemia) HRX gene on chromosome 11q23 in 50% of cases of congenital leukemia (both ANLL and ALL), and this is an unfavorable prognostic sign at all ages. Despite its unfavorable prognosis, ANLL may sometimes regress, though it may recur later on. It is important to distinguish congenital leukemia from florid neonatal leukemoid reactions, which are benign conditions defined as "transient abnormal myelopoieses", or "transient myeloproliferative disorders" (TMD), and characterized by hepatosplenomegaly and the presence of myeloid and lymphoid immature cells. The differential diagnosis must also consider fetal erythroblastosis and viral and bacterial infections. TMD differs from congenital leukemia in that the hemoglobin and platelet counts are normal, and blasts in the bone marrow are less than 15%, despite large numbers of white cells and myeloblasts in the peripheral blood. In these benign forms, the newborn's general condition is good and no specific therapy is required. In TMD, hematological indices return to normal over a period of time that may last from a few weeks to 1–2 months. Treatment guidelines for neonatal leukemia are the same as for older children, paying appropriate attention to the dosage of drugs involved, which must be administered in specialized hematological departments. Where feasible, high-dose therapeutic programs followed by hematopoietic cell transplants improve the remission and survival rates, which are currently about 20% [21–24].

111.2.8 Retinoblastoma (RB)

Newborn babies with retinoblastoma lack the normal "red reflex" when undergoing ophthalmoscopic examination, which is a part of routine newborn screening. A family history of retinoblastoma mandates a complete ophthalmic investigation because of the risk of bilateral disease. Familial cases (40%) are caused by a hereditary germinal mutation of the RB1 gene on chromosome 13q14. In such cases, DNA analysis identifies the individuals at risk. Hereditary cases are more common in the newborn, while non-hereditary, unilateral cases are seen more often in older children, who have a "de novo" RB gene mutation. Therapy is based on the outcome of a complete ophthalmic examination and MR can determine the extent of the lesion. The extent of retinal involvement is decribed as a percentage of the total retinal area. For localized forms, laser therapy and cryotherapy, combined with CHT using cisplatin and vepesid, are associated with the best prognosis for vision and survival. Surgery is limited to conditions affecting the extraocular areas, while radiotherapy (which was once used to prevent enucleation) has been abandoned because of the risk of secondary radio-induced tumors in adulthood. Therapy must be coordinated by oncologists and specialized ophthalmologists [2, 25].

111.2.9 Very Rare Congenital Tumors

111.2.9.1 Melanocytic Neuroectodermal Tumor (Melanotic Progonoma)

This tumor of neuroectodermal origin is characteristically located in the jaw, while other locations include the mediastinum and brain. It may be malignant and produce metastases. Surgery and chemotherapy are used, depending on the site of the lesion and the mitotic index.

111.2.9.2 Congenital Melanocytic Nevi

This condition is found in 1% of newborns. Giant forms are extremely rare and may contain nodular areas with a different mesenchymal cellular component. They sometimes resemble melanomas. Giant nevi undergo malignant transformation in

2–5% of cases. Fatal cases of congenital melanoma, with an intracranial involvement, can also occur.

111.2.9.3 Langerhans Cell Histiocytosis (LCH)

LCH comes half-way between malignant and histiocytic disorders. It affects one in a million newborns and 60% are cases of disseminated disease (with multiple organ involvement) while 40% are only cutaneous conditions. Gastrointestinal disease causes diarrhea, vomiting, and protein-losing enteropathy. The diagnosis of LCH requires a positive outcome of the CD1a immunohistochemical test. Depending on the extent of the disease, treatment may include steroids and vinblastine, with or without methotrexate.

The survival rate is 50% in cases of multiple-organ involvement, while it is more than 90% in patients with isolated disease [5, 26].

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Fetal Infections: Cytomegalovirus, Herpes Simplex, and Varicella

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112.1 Introduction

This chapter reviews three herpes viruses that cause infections of the fetus and/or newborn. These are herpes virus V, also called cytomegalovirus (CMV), herpes virus I and II, also called herpes simplex virus 1 and 2 (HSV), and herpes virus III, also called varicella-zoster virus (VZV). With the possible exception of HSV, CMV and VZV may produce severe fetal disease following a primary maternal infection during pregnancy when, in the absence of maternal immunity, these organisms are carried in the bloodstream to the placenta and then on to the fetus. With CMV and VZV, primary maternal infection during pregnancy does not always result in intrauterine infection of the fetus; and when intrauterine infection does occur, severe fetal disease does not always follow. Most commonly, infants infected in utero with CMV appear normal at birth. Chronic persistent infection with each of these herpes viruses causes progressive disease with significant developmental abnormalities which become apparent over the first several years of life. In general, infection of the mother with these viruses and the development of immunity prior to conception protects the fetus either from infection or from the severe disease both in utero and after birth for few months. With CMV, for example, women immune to the virus prior to pregnancy may deliver infants with intrauterine acquired infection but, with rare exceptions, congenital cytomegalic inclusion disease occurs only as the result of a primary infection during pregnancy.

Along with maternal immunity, another important factor that affects both the frequency of transplacental transmission and the severity of disease, is the gestational age of the fetus when the woman becomes infected. Infections with HSV 2 are for most part acquired during delivery from virus in the maternal cervical-vaginal tract. Maternal immunity to HSV 2 prior to delivery protects the newborn from severe or fatal disease due to HSV infection of the newborn. HSV 1 is a virus which is usually orally transmitted but may infect newborns and produce severe disease in the absence of maternal immunity.

Another influence on the severity and the manifestations of an intrauterine infection is the tissue tropism of each virus. Tissue tropism is the affinity of a particular microbe for specific cell types or tissues. Most of the microbes causing intrauterine infection replicate in all organs and tissues, hence causing disease in any or all organs or tissues. An example is VZV which is tropic for neural tissue, and many of the manifestations of severe intrauterine infection with VZV arise from effects upon the developing nervous system.

112.2 Cytomegalovirus

Human cytomegalovirus (CMV) contains within its capsid a double stranded DNA molecule of 150 million molecular weight. This DNA molecule contains at least several hundred genes and is the largest genome of any known virus. CMV viral particles are structurally similar to other human herpes viruses. The virus has a 65 nanometer inner core containing the viral DNA. The inner core is within an icosahedral protein capsid comprised of 162 capsomeres. This is in turn surrounded by a tegument layer and an outer enveloped membrane containing glycoproteins. The envelope glycoproteins are antigenic and are responsible for generating an immune response. The majority of the neutralizing antibodies induced by CMV antigens are directed against the major CMV glycoproteins. Cellular immune responses such as cytotoxic T-cells are directed primarily against the major tegument protein, pp65. There is only a single serotype of CMV. Thus antibodies induced by one viral isolate cross-react with most or all epidemiologically unrelated isolates. Genetically, however, there are probably thousands of different isolates. Each isolate of CMV differs genetically from all other epidemiologically unrelated isolates. CMV infects nearly all humans

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