Phagocytes Defects

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Core Messages

- Severe congenital neutropenia is a syndrome due to various gene defects rather than an isolated disease entity; there are still a number of patients with no underlying gene defect.
- Complete blood cell count and differential formula, twice weekly over 6 weeks, and antineutrophil antibodies are essential part of the 'workup' for patients with neutropenia.
- In the patients with wounds without pus, the diagnosis of leukocyte adhesion deficiency should be considered as well as RAC-2, beta-actin and specific granule deficiency.
- Mouth and teeth should be examined in the patients with phagocytes defects.
- Late presentation of chronic granulomatous disease – 'mild' or dramatic – does occur more often than we think.
- Think of other syndromes where neutropenia may be a part of the clinical presentation.

4.1 Introduction

Our understanding of primary immunodeficiency diseases (PID) in general is changing, shifting from the conventional towards more complex and not only immune system defined [38]. As with other PID, the recent progress in molecular biology over the last decade made an impact of our better understanding of the nature of phagocytes defects (Table 4.1). Fifty years after the description by Kostmann, a gene mutation has been identified in patients with the syndrome bearing his name [35, 37].

Whilst long-term follow up of relatively large patient groups with known gene mutation(s) (thanks to international multi-centre studies) [113] will give us more insight into the natural course of these diseases and will influence our treatment approaches in the future, the clinical identification and careful description of isolated patients will continue to add to our better understanding of the disease process. In that context and related to the clinician seeing a patient, the ESID (European Society for Immunodeficiencies)/PAGID (Pan-American Group for Immunodeficiency) diagnostic criteria for severe congenital neutropenia (SCN) from 2006 could be an example: as commonly perceived, SCN is an isolated condition due to several gene mutations, but it is as well a part of many complex syndromes [58].

Another half-century worth mentioning is the 'coming to age' of hematopoietic stem cell transplantation (HSCT) [7], still the curative procedure for most phagocyte deficiencies. The future looks even better for these patients as the era of gene therapy has arrived, albeit still controversial, with (un)expected complications and thus as yet far from being 'the perfect treatment' [140].

4.2 Severe Congenital Neutropenias *(ELA2 Deficiency, GFI1 Deficiency, HAX1 Deficiency, CSF3R Deficiency, Neutropenia with Myelodysplasia)*

4.2.1 Definition

Severe congenital neutropenia (SCN, OMIM#202700) is a rare primary immunodeficiency disease with an estimated frequency of 1–2 cases per 10^6 population [150, 175, 191]. SCN is characterized by early onset severe bacterial infections and persistent severe neutropenia [149, 150, 191, 199, 200]. Rolf Kostmann described this disorder for the first time in 1956, in a Swedish family with severe bacterial infections and severe neutropenia, which was characterized by a maturation arrest of myeloid differentiation at the promyelocyte–myelocyte stage [91, 92].

Table 4.1 Characteristics of phagocytes defects

Diseases	Genetic defects	Inheritance	Associated features
Severe congenital neutropenias (SCN)	*ELA2*	AD	Myelodysplasia
	GFI1	AD	B/T lymphopenia
	HAX1	AR	Increased immunoglobulin levels
	GCSFR	AD	G-CSF refractory neutropenia
	WASP	XL	Monocytopenia; myelodysplasia
Cyclic neutropenia	*ELA2*	AD	Oscillations in production of all types of blood cells
Leukocyte adhesion deficiency (LAD)	*ITGB2*	AR	Delayed umbilical cord separation; omphalitis; skin ulcers; leukocytosis
	FUCT1	AR	Periodontitis; mental retardation; hh blood group
	CalDAG-GEFI	AR	Delayed umbilical cord separation; skin ulcers; bleeding disorder
RAC-2 deficiency	*RAC2*	AD	Poor wound healing; leukocytosis
β-Actin deficiency	*ACTB*	AD	Mental retardation; photosensibility
Chronic granulomatous disease (CGD)	*CYBB*	XL	Immune dysregulation diseases (inflammatory bowel disease, genitourinary tract strictures, chorioretinal scars); McLeod syndrome (in patients with intertitial deletions); discoid lupus and oral ulcers (in female carriers)
	CYBA	AR	Immune dysregulation diseases
	NCF1	AR	(less frequently than XL-CGD)
	NCF2	AR	
Neutrophil G-6PD deficiency	*G6PD*	XL	Hemolytic anemia
Myeloperoxidase deficiency	*MPO*	AR	Asymptomatic; candidiasis
Specific granule deficiency	*CEBPE*	AR	Bilobed nuclei of the neutrophils
Shwachman–Diamond syndrome	*SBDS*	AR	Exocrine pancreatic insufficiency; Chondrodysplasia
Localized juvenile periodontitis	*FRP1*	AR	Aggressive periodontitis
Papillon-Lefèvre syndrome	*CTSC*	AR	Periodontitis; palmoplantar hyperkeratosis

4.2.2 Etiology

The pathophysiology and underlying genetic defect of SCN is not completely understood. The current knowledge on its pathophysiology shows that it is a multigene disorder with a common hematological and clinical phenotype [175]. Congenital neutropenia is genetically heterogeneous, and different forms of inheritance, including autosomal recessive, autosomal dominant, X-linked and sporadic forms, were reported [6, 18, 25, 70, 124, 150, 191] (Table 4.2). Considering this genetic heterogeneity of SCN, it seems that several pathologic mechanisms may lead to such phenotype due to downregulation of common myeloid transcription factors [175]. Absence of lymphoid enhancer-binding factor 1 (LEF1) could be an important patho-

Table 4.2 The current understood genetic defects associated with congenital neutropenias

Diseases	Genetic defets	Inheritance	Associated features	Further details
Severe congenital neutropenia	*ELA2*	AD	Myelodysplasia	Sect. 4.2
	GFI1	AD	B/T lymphopenia	Sect. 4.2
	HAX1	AR	Increased immunoglobulin levels	Sect. 4.2
	GCSFR	AD	G-CSF refractory neutropenia	Sect. 4.2
X-linked neutropenia/ myelodysplasia	*WASP*	XL	Monocytopenia, myelodysplasia	Sect. 4.2
Neutrophil G-6PD deficiency	*G-6PD*	XL	Hemolytic anemia	Sect. 4.8
Shwachman–Diamond syndrome	*SBDS*	AR	Exocrine pancreatic insufficiency, chondrodysplasia	Sect. 4.11
p14 deficiency	*P14 (MAPBPIP)*	AR	Partial oculocutaneous hypopigmentation, short stature	Sect. 5.3
Chediak-Higashi syndrome	*LYST*	AR	Partial oculocutaneous hypopigmentation, giant lysosomes, defective NK and T lymphocytes activities	Sect. 5.3
Griscelli syndrome, type 2	*RAB27A*	AR	Partial oculocutaneous hypopigmentation, defective NK and T lymphocytes activities	Sect. 5.3
Hermansky–Pudlak syndrome, type 2	*AP3B1*	AR	Oculocutaneous hypopigmentation, defective NK and T lymphocytes activities	Sect. 5.3
WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome	*CXCR4*	AD	Decreased immunoglobulin levels, reduced B cell number, warts	Sect. 6.5
Immunoglobulin class switch recombination deficiencies (affecting CD40-CD40L)	*CD40L, CD40*	XL, AR	Increased or normal IgM level, decreased other isotypes	Sect. 2.8
Cartilage hair hypoplasia	*RMRP*	AR	Short-limbed dwarfism with metaphyseal dysostosis	Sect. 9.6
Glycogen storage disease Ib	*G-6PT1*	AR	Hypoglycemia	Sect. 10.5.5
Barth syndrome	*TAZ1*	XL	Short stature, skeletal myopathy, dilatative cardiomyopathy	Sect. 10.5.6
Dyskeratosis congenita	*DKC1*	XL	Reticulate skin pigmentation, nail dystrophy, leukoplakia of the oral mucosa, aplastic anemia	Sects. 9.9 and 10.3.11

logic mechanism, irrespective of mutation status [174, 175].

While mutations in the gene encoding neutrophil elastase (*ELA2*, OMIM*130130) is the underlying genetic defect in more than half of SCN cases with autosomal dominant and sporadic forms [49, 158, 191], mutations in the gene encoding HCLS1-associated protein X1 (*HAX1*, OMIM*605998) were identified in patients with autosomal recessive SCN [89, 150], also known as Kostmann Syndrome (OMIM#610738). Heterozygous mutations in the protooncogene growth factor-independent 1 (*GFI1*) gene (OMIM*600871), which targets *ELA2*, also cause an autosomal dominant form of SCN [137]. X-linked form of congenital neutropenia (OMIM#300299) can be caused by a constitutively activating mutation in the *WAS* gene (OMIM*300392), which is also mutated in the Wiskott-Aldrich syndrome [50] (see Sect. 9.4 for more details).

Neutrophil elastase protein has a role in synthesizing the promyelocytes [8] and *HAX1* has a role in

controlling the apoptosis [43]. Mutant *HAX1* and also *ELA2* could accelerate apoptosis in myeloid progenitor cells of the patients [8, 36, 48].

Despite of discovering the mutations of such genes in SCN, there are still SCN patients without defined gene mutations [25, 162]; so, future genetic studies should be performed to discover other responsible genes in controlling the survival of neutrophils in these patients.

4.2.3 Clinical Manifestations

Early onset recurrent bacterial infections are the hallmark of SCN. The patients usually experience such infections by the age of 1 year. The most common presenting features are superficial abscesses, oral ulcers, cutaneous infections, omphalitis, pneumonia, and otitis media [149, 150, 191]. During the course of the disease, the patients usually develop abscesses in different sites, mucocutaneous manifestations, respiratory infections, and diarrhea [147, 149, 150]. Frequent aphthous stomatitis and gingival hyperplasia lead to loss of permanent teeth in childhood [191]. Recently, neurological disorders, including developmental delay and epilepsy, are reported in some SCN patients with *HAX1* mutations [36, 146].

Increased serum immunoglobulin levels is a common finding in SCN patients, which may be secondary to recurrent infections or due to a possible effect of the gene defect in both myelopoiesis and lymphopoiesis [150, 191].

It is estimated that splenomegaly could be detected in one-fifth of SCN patients before treatment with granulocyte colony-stimulating factor (G-CSF) and up to half of them through 10 years of treatment [191]. SCN is also considered as a preleukemic syndrome. While a number of SCN patients are complicated with myelodysplastic syndrome and acute myeloid leukemia during their disease [157, 175, 191], the presence of these complications has a high correlation with occurrence of acquired mutation in the granulocyte colony-stimulating factor receptor (*GCSF-R*) gene (OMIM*138971). Such mutations are detected in approximately 80% of the SCN patients who developed acute myeloid leukemia [52, 175].

4.2.4 Diagnosis

Presence of neutropenia in association with early onset severe and recurrent infections should raise suspicion of SCN, especially in those with superficial abscesses and oral ulcers. In fact, the presence of abscesses, ulcers and gingivitis implies clinically significant neutropenia [149]. Timely referral to a hematologist and/or clinical immunologist remains the key to the successful diagnosis and further management of patients with SCN, as delay in both reaching the diagnosis and starting the appropriate treatment increases the mortality in childhood [145, 150].

SCN patients typically have persistent severe neutropenia of less than 500/mm^3, and increased susceptibility to recurrent severe bacterial infections from early infancy. In addition to performing serial complete blood count (CBC) in order to determine the chronicity and severity, other causes of secondary neutropenia should be excluded. Review of the clinical history is important to rule out drug exposure and underlying illness such as autoimmune diseases [149]. CBC often indicate increased number of platelets, monocytes, and eosinophils, while mild anemia is usually seen [175].

Immune neutropenia of infancy should be excluded by testing for the presence of antineutrophil antibodies [191]. When antineutrophil antibody mediated neutropenia is present in the newborn period, the antibodies generally are not a result of autoimmunity as it is in older children and adults, but are usually of maternal origin, arising from maternal–fetal incompatibility at neutrophil-specific antigen loci. Many of these neutrophil specific antigens are expressed on the antibody Fc receptors of neutrophils. Maternal mediated immune neutropenia is a self-limited process that will improve over several months as maternal antibodies are cleared, and should be managed conservatively.

Bone marrow examinations of the patients with SCN usually show a maturation arrest of neutrophil precursors at an early stage (promyelocyte-myelocyte) [6, 149, 150, 191, 199, 200] (Fig. 4.1). Cellularity is usually normal or a little decreased, while increased number of eosinophils and monocytes is often detected in the bone marrow [191].

Molecular studies could confirm the definite diagnosis in SCN patients and are also useful as predictors for response to treatment and outcome. However, the diagnosis of SCN rests primarily on the clinical features of the disease and peripheral blood studies [149].

4.2.5 Management

In the absence of appropriate treatment, affected children suffer from life-threatening infections [36, 149, 175, 191, 198]. Bone marrow transplantation (BMT) used to be the only treatment option until 20

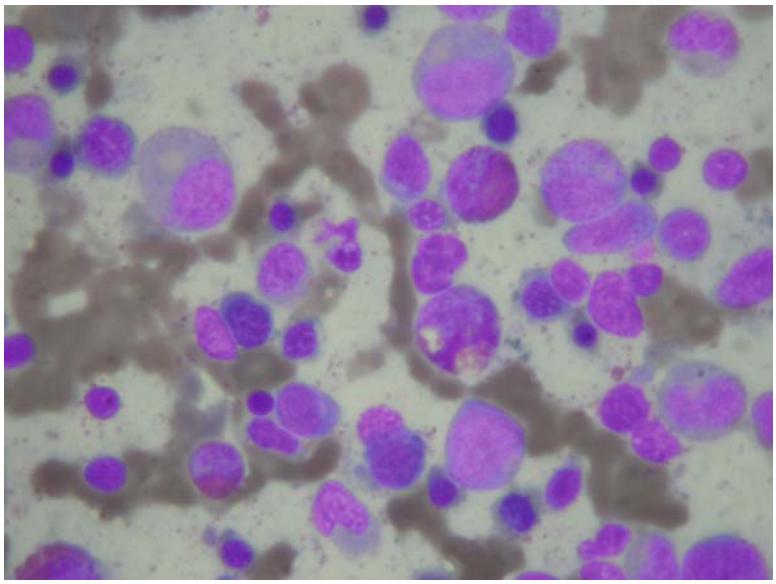

Fig. 4.1 The bone marrow morphology of a patient with severe congenital neutropenia

years ago. Since G-CSF therapy became available as a treatment option for SCN, it has become possible to manage most patients without a requirement for BMT. G-CSF therapy has made considerable impact towards prognosis and quality of life of these patients [26, 36, 150, 191, 198, 200]. Recombinant G-CSF is the first choice of treatment for the SCN patients and more than 90% of the patients respond to G-CSF administration, which increase the number of neutrophils and consequently reduce the number of infections and days of hospitalization [175, 191]. However, in patients with mutations in the *G-CSFR* gene who do not respond to G-CSF treatment, and in those with continuing severe bacterial infections or complicated with development of myelodysplasia, HSCT using either bone marrow, mobilized peripheral blood stem cells, or cord blood as the source of allogeneic stem cells would be the treatment of choice [201].

It is recommended that all SCN patients should be followed up at least twice per year and CBC should be performed at least every 3 months [191].

4.3 Cyclic Neutropenia

4.3.1 Definition

Cyclic neutropenia (OMIM#162800) is a rare primary immunodeficiency disease with an estimated frequency of 1 case per 10^6 population, characterized by neutropenia occurring every 3 weeks and lasting for 3–6 days [45, 47, 59, 148, 149, 151]. Dr. Leale described this disorder for the first time in 1910, in an infant with recurrent episodes of fever, skin infections, stomatitis, and neutropenia [97]. Patients with cyclic neutropenia are usually asymptomatic. However, they can frequently suffer from severe bacterial infections, oral lesions and cutaneous manifestations during the episodes of neutropenia [59, 148, 149, 151].

4.3.2 Etiology

Cyclic neutropenia is an autosomal dominant or sporadic disease, due to the periodic failure in production of granulocytes, presumably at the stem cell level [148]. The pathophysiology and the affected function in this disease has not been fully understood, but it seems that cyclic neutropenia is due to an abnormality in the regulation of early hematopoietic precursor cells [148, 149]. It could lead to oscillations in production of all types of blood cells. Neutropenia and leukopenia occur together in most situations, and cyclic (and for some nonneutrophil lineages counter-cyclic) fluctuations of monocytes, eosinophils, lymphocytes, platelets, and reticulocytes are also reported [45, 47, 148, 149, 151]. Mutations in the *ELA2* gene (OMIM*130130) are reported as the underlying genetic defect in several patients with cyclic neutropenia [8, 48, 49, 70, 124, 158]. It is also important to distinguish

the congenital autosomal dominant form of cyclic neutropenia from acquired cyclic neutropenia that may complicate the clinical manifestations of benign and leukemic expansions of large granular lymphocytes [18]. Generally, congenital cyclic neutropenia is characterized by extremely regular cycles of almost exactly 21 days duration, while acquired cyclic neutropenias may have irregular cycles and/or cycles significantly different from 21 days duration. It is important to note, however, that administration of G-CSF to patients with congenital cyclic neutropenia may significantly alter the cycle duration in some patients.

4.3.3 Clinical Manifestations

Patients with congenital cyclic neutropenia are generally healthy between neutropenic periods, but during the episode of neutropenia suffer aphthous stomatitis, oral ulcers, gingivitis, abscesses and occasionally overwhelming bacterial infections [48, 59, 148, 149, 151]. The symptomatic episodes of fever and infections usually recur approximately every 3–4 weeks. The neutropenic periods are associated with infections especially in oral cavity and mucous membranes, where oral ulcers and periodontitis are common. Cutaneous infections, upper respiratory infections and skin abscesses are also common. Perirectal and genital areas are susceptible to recurrent infections and abscesses [45, 47, 148, 149, 151]. Because many patients with congenital cyclic neutropenia tend to be clinically well between nadirs, it is easy to miss the early signs of the particularly life-threatening danger to these patients of the development of necrotizing enterocolitis (typhlitis), which may rapidly progress to acute perforation of the bowel with bacteremia and septic shock.

4.3.4 Diagnosis

Congenital cyclic neutropenia is diagnosed by documenting the very regular periodic oscillations in the circulating neutrophil count from normal to neutropenic levels through at least a 3-week period, lasting for 3–6 days [45, 47, 59, 148, 149, 151]. In patients with neutropenia, the clinical history and examination of the peripheral blood smear are the most important aspects of the diagnostic evaluation. Examination of the oral cavity, perianal region, and skin is necessary in order to assess the clinical impact of neutropenia [148, 149]. As previously noted, in patients not being treated with G-CSF, the period of cycling is generally very regular and most often is close to 3 weeks duration. However, the cycling periodicity can vary somewhat from a patient to another patient and can be altered by administration of G-CSF. It is recommended that for diagnosis a CBC with assessment of differential lineages be performed at least twice or even three times weekly over 6–9 weeks to document the typical cyclic pattern of neutropenia [148]. In sporadic cases where a family history is absent, evidence for cycling from early childhood is absent, and/or the cycling is erratic or very different from 21 days, acquired cyclic neutropenia must be considered in the differential diagnosis.

Bone marrow examination of the patients during the neutropenic period shows maturation arrest of neutrophil precursors at an early stage, but is not a necessary investigation in every patient [59, 148, 151].

4.3.5 Management

The quality of life and life expectancy of the patients with congenital cyclic neutropenia are good, if patients are diagnosed and followed regularly by attentive physicians and dentists [148, 149, 151]. Although the prognosis is good with a benign course, approximately 10% of patients may experience life-threatening infections. Besides prophylactic antibiotics, in some patients treatment with recombinant human G-CSF in anticipation of and into the time of 'cycling nadir' may be all that is needed only over a period of several days to sufficiently increase blood neutrophil counts to achieve reduction in infection rate, and improvement in survival and quality of life [45, 46].

4.4 Leukocyte Adhesion Deficiency *(LAD Types 1–3)*

4.4.1 Definition

Leukocyte adhesion deficiency (LAD) is a rare primary immunodeficiency disease with an estimated frequency of less than 1 case per 10^6 population. LAD is caused by

a defect in neutrophil adhesion and characterized by skin ulcers, poor wound healing and recurrent bacterial infections [107]. It was first described in late 1970s, in patients with delayed umbilical cord separation, recurrent bacterial infections, and defective neutrophil mobility [81]. Further reports demonstrate that there are at least three genetic forms of LAD, though one form affecting leukocyte beta integrins is by far the most common [107].

4.4.2 Etiology

Normal inflammation is characterized by the accumulation of leukocytes at the involved site due to transmigration of blood leukocytes through the endothelial barrier. The migration of phagocytic cells out of the blood vessels (diapedesis) into the extravasular space at sites of inflammation and infection depends on a variety of adhesive molecules. In a temporal order, first selectins are necessary to make the transient low adhesive strength attachments of leukocytes to the endothelium that occur in a process that has been termed "rolling". Rolling leukocytes come to a stop and spread out over the endothelial cell when integrins composed of a common beta-chain (CD18) and variable alpha-chains lymphocyte function-associated antigen-1 or LFA-1 (CD11a), macrophage antigen-1 or Mac-1 (CD11b) and P150,95 (CD11c) are increased in number at the cell surface and activated to undergo structural conformational changes that cause them to adhesively interact strongly with the intracellular adhesion molecules ICAM1 and ICAM2, present on the endothelium and other leukocytes. The intercellular adhesion molecules (ICAM) are themselves also activated to more strongly interact with integrins by local inflammation. Endothelial cells also express selectins which also participate in the enhancement of binding. Binding of the integrins to the ICAM molecules are firm enough to withstand the continuous shear forces of the blood flow. In order for these strongly adherent leukocytes to migrate between endothelial that actually weaken the integrin-ICAM bonding to allow migration, the migration through the sub-endothelial membranes is dependent on the Ig-superfamiliy platelet cell adhesion molecule (PCAM)-1. Thus, a highly choreographed temporal series of changes in adhesion molecules is required for the entire process of diadepesis to occur. The common problem of all leukocyte adhesion deficiency syndromes is that the leukocytes cannot leave the blood vessels to migrate normally into the inflamed tissues because one part or another of this process is abnormal (Fig. 4.2) [107].

The underlying causes of these different diseases are either deficiencies or defects of the adhesion molecules. Up to now, three different syndromes have been delineated (Table 4.3). LAD type 1 (LAD-1) (OMIM#116920), by far the most common form of LAD, is caused by a defect of the common chain of the beta 2 integrin family (*ITGB2* or *CD18*) (OMIM*600065). LAD type 2 (LAD-2) or congenital disorder of glycosylation type IIc is caused by mutations in the solute carrier family 35, member C1 (*SLC35C1*) gene (OMIM#266265), which encodes GDP-fucose transporter 1 (FUCT1). It leads to an absence of fucosylated carbohydrate ligands resulting in defective rolling [61, 63]. The impaired fucosylation impairs transport of fucose via the GDP fucose transporter into the Golgi apparatus [102, 105, 194]. Recently, a novel defect was described in which a truncation affected both localization of the fucose transporter into the Golgi as well as enzymatic activity [82].

The third type of LAD (LAD-3) has recently been described which appears to be result from failure of cytokine activation of a number of integrins [3, 60]. The actual molecular defect is not fully understood at the genetic level, but an abnormality of Rap1 function, a regulatory GTPase, seems to be involved in regulation of integrin signaling [88]. Recently, the LAD-3 phenotype was introduced in mice lacking the signaling molecule calcium-diacylglycerol guanine nucleotide exchange factor I (*CalDAG-GEFI*) [17]. This signaling molecule controls the activation of integrins in the hematopoietic system. So, neutrophils of mice lacking such molecule exhibited strong defects in Rap1 and beta 1 and 2 integrins activation, which results in failure of adhesion and migration into inflammation sites. As CalDAG-GEFI regulates the activation of beta 1 and 3 integrins in platelets, mice lacking this molecule have complete inhibition of arterial thrombus formation [17].

4.4.3 Clinical Manifestations

LAD-1 is characterized by delayed separation of the umbilical cord (normal separation is 3–45 days with a mean of 10 days) with concurrent omphalitis (Fig. 4.3), recurrent deep bacterial infections of the skin, periodontitis, very characteristic absence of pus formation and impaired wound healing. Infections are frequently caused by *Staphylococcus aureus* or gram-negative bacilli. The absence of pus formation at the site of infection is a hallmark of LAD-1. Without appropriate therapy more than 75% of the patients die before their 2nd birthday [59].

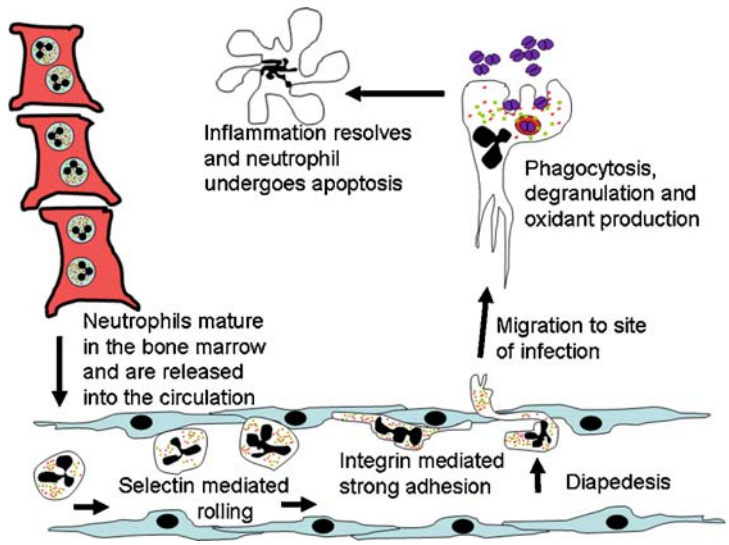

Fig. 4.2 The life cycle of the neutrophil is shown, including the phases of migration of neutrophils to sites of infection or inflamed tissues. Neutrophils develop in the bone marrow (*upper left*) and are released into the circulation. Neutrophils sense infection or inflammation in the post capillary venule (*bottom of figure*) where bacterial factors and inflammatory chemoattractants and chemokines act on both the neutrophils and endothelial cells to increase adhesion. The initial phase of increased adhesion engages selectins which mediate short-lived weak binding encounters between neutrophils and endothelium (rolling). This is followed by activation of integrins, triggering strong adhesive forces that mediate spreading of neutrophils onto the endothelium. This is followed by additional conformational changes that weaken integrin adhesion, allowing chemotactic migration of the neutrophil between endothelial cells (*lower right*), though the basement membrane, and into the tissues to the site of infection. At the site of infection neutrophils phagocytose bacteria (*upper right*) or other pathogens, triggering the process of degranulation, production of reactive oxygen species, and activation of proteases. Over hours to days, neutrophils proceed into an apoptotic phase (*upper middle*), triggering engulfment by macrophages in a process that minimizes tissue damage and leads to resolution of inflammation

Table 4.3 Characteristics and treatment of leukocyte adhesion deficiencies (LAD)

Type	Characteristics	Molecular defect and diagnosis	Therapeutic options
LAD-1	Delayed separation of the umbilical cord, omphalitis, skin ulcers, leukocytosis	Common chain of beta integrins, CD18 absent	*For acute infections*: Antibiotics, granulocyte transfusions *Curative treatment*: Hematopoietic stem cell transplantation *Future option*: Gene therapy
LAD-2	Periodontitis, mental retardation, short stature, hh blood group	Impaired fucosilation, mutations in the GDP-fucose transporter, leukocytosis, Bombay blood group, absence of SLEX (CD15a)	Antibiotic prophylaxis (e.g. Cotrimoxazol), good dental hygiene, early fucose supplementation
LAD-3	Same as LAD-1, bleeding disorder	Normal CD18 expression, SDF-1 activation of both VLA-4 and LFA-1 avidity to ligand impaired, decrease of RAP-1	See LAD-1

Infections in patients with LAD-2 are, in general, less severe than in patients with LAD-1. There is no delay in the separation of the umbilical cord, pus formation is impaired but not absent, and skin, lung or periodontal infections are generally not life threatening. The severity of infections may decrease over time and adults frequently suffer primarily from periodontitis. A hallmark of LAD-2 is severe delay in psychomotor development, microcephaly and cortical atrophy, and short stature [61, 63].

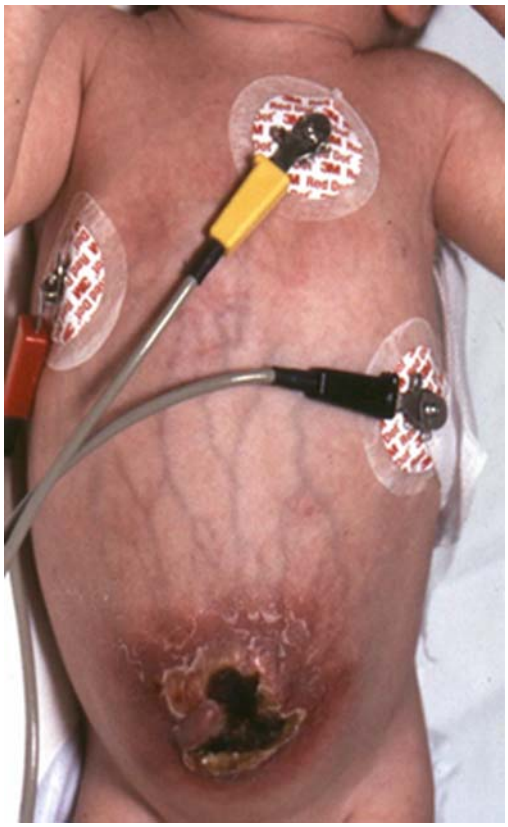

Fig. 4.3 Severe omphalitis in a child with LAD-1. (Courtesy of R. Seger; Zurich, Switzerland)

The clinical presentation of LAD-3 is similar to LAD-1, with severe recurrent bacterial infections, delayed separation of the umbilical cord and bleeding of the skin and mucous membranes [60].

4.4.4 Diagnosis

A combination of early bacterial infections, particularly omphalitis, with absent pus formation and marked peripheral blood neutrophilia (up to 50,000–100,000/μl in the presence of infection or in the range of 15,000/μl in the absence of infection) should trigger awake the suspicion of LAD-1.

Confirmation of the diagnosis is achieved with flow cytometry analysis of the common beta chain and the alpha subunits using monoclonal antibodies against CD18 and CD11. Severe phenotype is associated with an expression of CD18 of less than 1–2% compared to normal controls, whilst patients with some surface expression of CD18 (>2–30 % of normal) may have a more mild to moderate clinical course. However, only 25% of patients with even the "milder" phenotype survive beyond 40 years [107]. These patients with milder phenotype often suffer from large nonhealing infected ulcers of the groin and lower extremities that respond poorly to antibiotics and eventually becoming colonized and infected with unusual organisms that are resistant to most antibiotics.

Carrier detection is possible by the demonstration of reduced expression of CD18 (40–60%) and prenatal diagnosis is possible by mutational analysis in families with previously diagnosed individuals [189]. Preimplantation genetic diagnosis represents an alternative to conventional prenatal diagnosis to enable carrier couples to achieve a healthy pregnancy. In a recent report, a multiplex fluorescence-based polymerase chain reaction assay was used to detect two different LAD-1 mutations in the CD18 gene [101]. The couple had previously conceived a daughter with LAD-1 who was a compound heterozygote (mother G400A substitution, exon 4; father C562T substitution in exon 5 of CD18). A cleavage-stage biopsy was performed on day 3 after standard in vitro fertilization and genetic analysis of blastomeres for the two mutations was carried out. After genetic analysis, three embryos were found to be unaffected, and two embryos were transferred, resulting in birth of an unaffected child. There are ongoing debates and possible ethical issues related to this procedure that will need to be clarified before it can be recommended as a 'standard approach'.

A diagnosis of LAD-2 should be suspected in a patient with the clinical features of psychomotor delay, and with recurrent but mild infections, leukocytosis and detection of the Bombay blood group (absence of H antigen) [67]. Peripheral blood leukocytes show an absence of Slex-expression (CD15A). Confirmation of the diagnosis needs sequence analysis of the GDP-fucose transporter. LAD-2 is therefore also considered as a congenital disorder of glycosylation and is classified as CDG-IIc [102, 103].

LAD-3 is a major differential diagnosis of LAD-1 and should be suspected in situations when CD18-expression in an otherwise 'typical LAD-1' patient is normal. The confirmation of diagnosis requires the demonstration of impaired integrin activation (e.g., activation of RAP-1) in specialized centres.

4.4.5 Management

In all three genetic types of LADs, infections should be treated promptly with appropriate antibiotics.

In LAD-1, transfusions of G-CSF stimulated granulocytes may be beneficial in severe infections, including the large chronic ulcers discussed above, that cannot be controlled otherwise. At the moment the only curative treatment for the severe phenotype is HSCT [181]. The survival rate was nearly 100% with HLA-compatible donors, compared to >80% when using HLA-nonidentical donors in this small series. The good outcome is possibly related to the importance of lack of beta-2 integrins (or their blockage) in enabling engraftment, as was demonstrated by using anti-LFA-1 antibodies to prevent graft rejection in T cell depleted haploidentical bone marrow transplantation for PID other than severe combined immunodeficiency (SCID) [65]. Besides classical myeloablative conditioning, nonmyeloablative regimens were tested experimentally in the canine model of LAD (CLAD) [14, 177]. In humans, recent evidence suggests that a nonmyeloablative conditioning regimen might provide a less toxic but similarly effective approach to LAD. However, the hypercellular marrow seen in LAD-1 patients may require more of a "reduced intensity" type of conditioning rather than the much less intensive regimens that are usually designated as "nonmyeloablative" to more reliably achieve clinically beneficial levels of engraftment. Rao et al. [143] reported 33 consecutive unrelated donor transplantations in children with PID, including 4 with LAD, using a reduced intensity conditioning regimen consisting of fludarabine 150 mg/m^2, melphalan 140 mg/m^2, and alemtuzumab (Campath 1H) 0.2 mg/kg/day for 5 days or antithymocyte globulin (ATG) 2.5 mg/kg/day for 4 days. Compared with a retrospective control cohort of 19 patients who underwent a myeloablative conditioning regimen, all children in both groups had primary engraftment and there was no statistical difference in the speed of immune reconstitution or incidence of graft versus host disease between the two groups. The overall survival in the group receiving the reduced intensity regimen was 94%, compared to 53% in the group treated with the myeloablative conditioning regimen. Studies of transplant in CLAD have indicated that significant clinical benefit may accrue even with only modest level of mixed myeloid lineage donor chimerism [14, 177].

In the future, an alternative therapy in patients without a suitable donor may be gene therapy, but thus far clinical trials using no marrow conditioning have failed to achieve long-term clinically beneficial levels of correction [16]. In the canine counterpart of this disease (CLAD), retroviral mediated gene transfer with nonmyeloablative conditioning resulted in a progressively increasing percentage of corrected cells over time. Bauer et al. evaluated ex vivo retroviral-mediated gene therapy in CLAD using 2 nonmyeloablative conditioning regimens – 200 cGy total body irradiation (TBI) or 10 mg/kg busulfan – with or without post-transplantation immunosuppression. In 6 of 11 treated CLAD dogs, therapeutic levels of CD18(+) leukocytes were achieved. Conditioning with either TBI or busulfan allowed long-term engraftment, and immunosuppression was not required for efficacy. The percentage of CD18(+) leukocytes in the peripheral blood progressively increased over 6–8 months after infusion to levels ranging from 1.26 to 8.37% at 1-year follow-up in the 6 dogs. These levels resulted in reversal or moderation of the severe CLAD phenotype and suggest that the corrected cells may even have a survival advantage, an important prerequisite of a successful gene therapy. Linear amplification-mediated polymerase chain reaction assays indicated polyclonality of insertion sites [15]. However, using retroviruses as gene vectors in gene therapy trials for SCID carries the risk of insertional mutagenesis [19, 79]. Therefore, new approaches are urgently needed.

Recently, the efficacy of a foamy virus vector (a type of lentivirus) expressing canine CD18 gene in CLAD was tested. Foamy viral vectors have broad host range, large capacity, efficacy in an overnight transduction protocol, and lower likelihood of activation of downstream genes than conventional murine retroviral vectors. Four of the five puppies receiving autologous, CD18 gene-corrected bone marrow CD34+ cells, preceded by a nonmyeloablative dose of 200 cGy total body irradiation, had complete reversal of the CLAD phenotype with 5–10% CD18 positive peripheral blood leukocytes 1 year after transplant. No adverse events like induction of leukemia have been observed, and all four animals have displayed polyclonality of foamy viral insertion sites [13, 107].

In the less severe phenotypes (CD18 > 2–30%) prophylactic antibiotics (e.g., Trimethoprim-Sulfamethoxazol 5 mg/kg once daily) are frequently sufficient to avoid severe infections, and careful oral hygiene is mandatory to prevent or ameliorate periodontitis.

In LAD-2 patients with recurrent infections antibiotic prophylaxis with Trimethoprim-Sulfamethoxazol TMP-SMX is beneficial. In some, but not in all patients, fucose supplementation may achieve a significant clinical improvement [62, 63, 114]. Therefore this modality should be tried in these patients. The initial dose is 25 mg/kg/bw 5 times a day, and this dose should be increased up to 500 mg/kg 5 times a day. Fucose supplementation should be started as early as possible to minimize neurological damage.

Therapy of LAD-3 is very similar to therapy of LAD-1 patients [3]. The only curative treatment is allogeneic HSCT.

4.5 RAC-2 Deficiency

4.5.1 Definition

RAC-2 deficiency or neutrophil immunodeficiency syndrome (OMIM#608203) is manifested by a severe defect in leukocyte migration. As in patients with LADs (see Sect. 4.4 for more details) and β-actin deficiency (see Sect. 4.6 for more details), there is lack of pus formation at the site of infection [5]. Ambruso et al. reported an infant with recurrent infections and poor wound healing, suggesting a neutrophil defect, in whom they found a missense mutation in the *RAC2* gene (OMIM*602049) [5].

4.5.2 Etiology

Ras-related C3 botulinum toxin substrate 2 or RAC-2 is a Rho-GTPase important for the expression of L-selectin, F-actin assembly, chemotaxis and superoxide generation and regulation of actin polymerization. In activated neutrophils, the cytosolic RAC-2 comigrates with $p67^{phox}$ (RAC-1 in macrophages) to attach to $p47^{phox}$ to form the NADPH oxidase complex (Fig. 4.4) [10]. The mutant RAC-2 does not bind to its physiological ligand GTP, thus activation of superoxid production via phagocyte oxidase is inhibited [125]. Because RAC-1 may partially substitute for RAC-2, the defect in oxidase activity is not as profound as seen with chronic granulomatous disease (see Sect. 4.7 for more details) and is affected by the stimulus used to activate the oxidase. Neutrophils from mice deficient in RAC-2 have defects in rolling on endothelium, chemotaxis and phagocytosis [153]. In humans with this disorder, neutrophils show also defects in chemotaxis, decreased release of enzymes of azurophilic granules after activation with N-formyl-methionyl-leucyl-phenylalanine (fMLP) or phorbol myristate acetate (PMA) and a deficient polarization and actin polymerisation in response to fMLP as well as the deficient production of reactive oxygen radicals in response to fMLP stimulation. Interestingly, as might be expected for a regulatory element important in several types of functional responses of neutrophils, the syndrome combines features seen in LAD, chronic granulomatous disease (CGD), specific granule deficiency (SGD) and β-actin deficiency. The *RAC-2* gene is located on chromosome 22q13 and has a size of 18 kb.

4.5.3 Clinical Manifestations

Patients with RAC-2 deficiency suffer from delayed separation of the umbilical cord, poor pus formation, non-healing perirectal/periumbilical abscesses, and peripheral blood leukocytosis similar to LAD-1.

4.5.4 Diagnosis

Wound biopsies show appropriate number of neutrophils which differentiate this disease from LAD-1. CD18 expression is also normal, while chemotaxis toward C5a, fMLP, and IL-8 is impaired. Moreover, neutrophil polarization in response to fMLP is also deficient. NADPH oxidase activity is normal after PMA, but decreased after fMLP stimulation [127]. The differential diagnosis to LAD-3 necessitates the demonstration of restoring oxidase activity in a cell-free system with addition of purified RAC-2.

4.5.5 Management

Infections should be treated with appropriate antibiotics and critical infections can be treated with granulocyte transfusions. Allogenic HSCT may be curative.

4.6 β-Actin Deficiency

4.6.1 Definition

β-actin deficiency is a leukocyte migration disease. As in patients with LAD, there is no pus formation at the site of infection.

4.6.2 Etiology

β-actin deficiency is an autosomal dominant deficiency of the actin polymerisation of neutrophils. A heterozygous negative dominant mutation of non-muscle β-actin (*ACTB*) (OMIM*102630) impairs the binding of profilin, which is an actin regulatory protein [126].

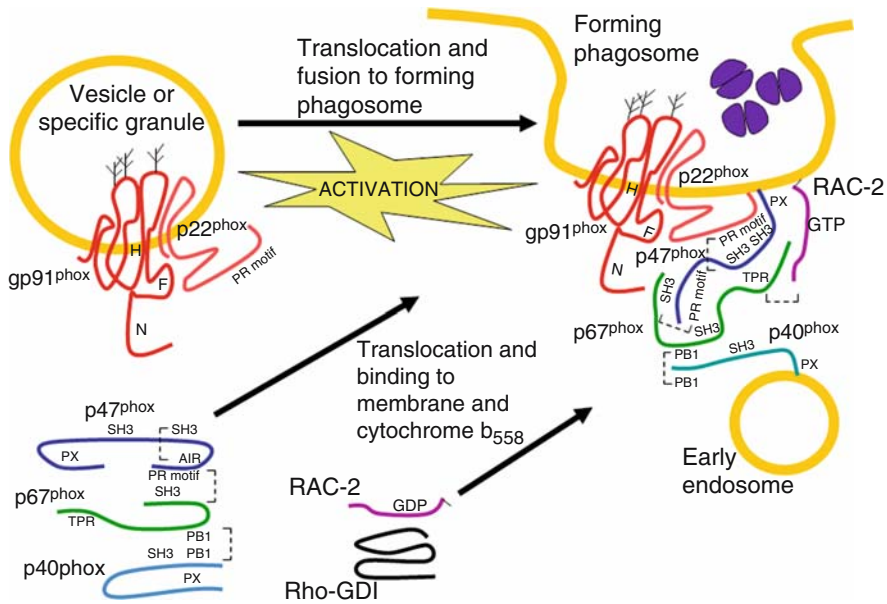

Fig. 4.4 Molecular features of activation mediated assembly of the phagocyte NADPH oxidase from subunit components are shown. The cartoon images of the subunits are highly schematized and not drawn to scale in order to emphasize some of the known structural features each subunit and some of the intra- and intermolecular binding affinities in the resting state (*left side* of figure) and upon assembly of the fully activated oxidase (*right side* of figure). Some known or suspected binding interactions between specific domain motifs are indicated by *dotted lines*. In the resting neutrophil the cytochrome b_{558} heterodimers consisting of gp91^{phox} and p22^{phox} are predominantly present in small vesicles and specific granules *(upper left)*. *N*, *F*, and *H* labels on the gp91^{phox} subunit indicate, respectively, the NADPH binding pocket and the Flavin binding site in the cytoplasmic C-terminal region, plus the two Heme moieties within the transmembrane region. Three n-glycosylation sites on two of the intravesicular (topologically extracellular) domains of gp91^{phox} are indicated by the small tree-like stick figures. Indicated in the C-terminal tail of the p22^{phox} subunit is a basic proline–arginine rich region (*PR* motif) capable of binding with a SH3 domain. In the resting neutrophil the p47^{phox}, p67^{phox} and p40^{phox} subunits exist in the cytoplasm predominantly as a heterotrimer, and the RAC-2 (RAC-1 in monocytes) exists separately in its unactivated inhibited GDP charged state bound to rho-guanine nucleotide dissociation inhibitor (*Rho-GDI*). Both p47^{phox} and p40^{phox} have PX domains at the N-terminal portion of the molecule that are protected by intramolecular interactions in the resting state, but which engage specific species of membrane lipid inositides in the activated cell. Of importance in the resting state is that a very basic autoinhibitory region (*AIR*) of p47^{phox} interacts with one of its own SH3 regions while a PR motif in a nearby domain binds to the C-terminal SH3 domain of p67^{phox}. Both p67^{phox} and p40^{phox} contain PB1 motifs that mediate binding between these two subunits, an interaction that also appears to stabilize and protect p67^{phox} from proteolysis. There is also some evidence to suggest that in the resting state, there is an additional intramolecular interaction between the PX domain and PB1 domain of p40^{phox} that inhibits and protects that PX group. Upon activation of the neutrophil, vesicles and specific granules containing membrane cytochrome b_{558} fuse with the forming phagosome (*upper right*), with early endosomes and/or at the plasma membrane. Phosphorylation of the AIR region of p47^{phox} disengages and unfolds it from the SH3 domain, leaving that SH3 domain free to interact with the PR motif of p22^{phox}. Other phosphorylation events induce additional conformational changes in p47^{phox} and p40^{phox} that enhance binding of PX domains to newly generated forms of membrane inositides. There is some evidence to suggest a distinct binding predilection of p47^{phox} or p40^{phox} PX domains, for the types of inositides appearing on activation in phagosome membranes and early endosome membranes, respectively (indicated schematically). Neutrophil activation also triggers disengagement of RAC-2 from the Rho-GDI with exchange of GDP for GTP allowing binding of RAC-2 to the TPR region of p67^{phox} and interaction of the RAC-2 myristoylated C-terminus with the membrane. The fully assembled oxidase shown schematically on the *right side* of the figure allows electrons to flow from NADPH through the flavin and heme moities to molecular oxygen to form superoxide in the phagosome

4.6.3 Clinical Manifestations

The patients suffer from recurrent bacterial and fungal infections without pus formation, mental retardation and photosensitivity. One patient developed recurrent stomatitis, cardiomegaly, hepatomegaly and hypothyroidism [125].

4.6.4 Diagnosis

Wound biopsies show reduced numbers of neutrophils. Chemotaxis and phagocytosis are markedly impaired as well as polymerisation of actin monomeres after activation. LAD-1 (CD18) and LAD-2 (CD15A) should be excluded. Definitive diagnosis can be achieved by mutational analysis of the *ACTB* (cytoplasmic actin) gene.

4.6.5 Management

HSCT is the therapy of choice to correct the immunodeficiency, but likely would not correct the associated nonhematologic abnormalities. Until transplant or if transplant is not possible, management with long-term prophylactic antibiotics should be instituted.

4.7 Chronic Granulomatous Disease (CGD) *(gp91 ^{phox} Deficiency, p22 ^{phox} Deficiency, p47 ^{phox} Deficiency, p67 ^{phox} Deficiency)*

4.7.1 Definition

Chronic granulomatous disease (CGD) is a genetically heterogeneous disease characterized by recurrent life-threatening infections with bacteria and fungi as well as dysregulated granuloma formation (excessive inflammation response). CGD is caused by defects in the NADPH oxidase, the enzyme complex responsible for the phagocyte respiratory burst which leads to the generation of superoxide and other reactive oxygen species (ROS) in phagocytic cells. The disease was first described by Janeway et al. in 1954 [86], but was not well characterized until 1959 by Bridges et al. [31]. It was initially termed fatal granulomatous disease of childhood, but with early diagnosis and better treatment the majority of patients can be expected to survive to adulthood and the prognosis no longer warrants that pessimistic name. The frequency of CGD in the general population is at least 1:200,000 live births, and likely higher than that [195].

4.7.2 Etiology

The fully assembled NADPH oxidase system is a six-protein complex. In the basal state, it exists separated into two compartments: a membrane-bound complex embedded in the walls of secondary granules and other vesicular structures, and proteins in the cytosol [165]. The secondary granule membrane and other vesicular structures contain the heme and flavin binding cytochrome b_{558}, composed of a 91-kd glycosylated β chain (gp91^{phox}) and a 22-kd nonglycosylated chain (p22^{phox}). The cytosolic components are p47^{phox}, p67^{phox}, p40^{phox} and RAC where much of the p47^{phox}, p67^{phox}, and p40^{phox} exists as an enzymatically inactive heterotrimeric complex separate from the RAC component (Fig. 4.4). With the exception of p40^{phox}, which is thought to have a regulatory role, all these components are necessary for the generation of superoxide.

After cellular activation, such as that initiated by phagocytosis of microbes, the cytosolic components p47^{phox} and p67^{phox} are phosphorylated. This results in a major conformational change in p47^{phox}, revealing new intramolecular sites that allow it to bind tightly to p67^{phox} and also to p22^{phox} at the cell membrane. RAC is myristoylated. Phosphorylated p47^{phox} and p67^{phox} in association with p40^{phox}, and the myristoylated RAC translocate to the forming phagosome membrane where they bind to the intracytoplasmic domains of the cytochrome complex (gp91^{phox} and p22^{phox}) to form the active NADPH oxidase molecular complex. Following assembly, electrons transfer from NADPH, passing first to the flavin domain and then the heme domain of the phagocyte oxidase, where it is finally donated to molecular oxygen, yielding the formation of superoxide (O_2^-). In the presence of superoxide dismutase, this is converted to hydrogen peroxide (H_2O_2), which, in the presence of myeloperoxidase and chlorine in the neutrophil phagosome, is converted to hypochlorous acid (HOCl), or bleach [165]. The rapid consumption of oxygen and production of superoxide and its metabolites is referred to as the respiratory burst, because the rate of consumption of oxygen and production of microbicidal oxidants that occurs in neutrophils peaks at about 10–15 min and then decreases significantly followed by a slow waning of the production over a prolonged period. It was previously thought that hydrogen peroxide and bleach were bactericidal per se. However, persuasive data from Reeves et al. showed that phagocyte production of the superoxide anion within the phagosome is electrochemically balanced by transport of very significant amounts of potassium ions into the phagosome. The K^+ likely acts to dissociate granule proteins from stabilizing matrix and together with the oxidants serves to activate proteases that have been released into the phagolysosome by the fusion of primary granules with the phagolysosome membrane upon cellular activation. In this paradigm, superoxide production serves

primarily to trigger the K^+ flux necessary to liberate and activate granule proteins including proteases, and not by its intrinsic microbicidal activity [144]. However, most investigators in the field continue to view the highly diffusible and reactive hydrogen peroxide generated from dismutation of superoxide as playing a significant role in antimicrobial killing.

Mutations in four genes account for all the known cases of CGD. The gene for $gp91^{phox}$, *CYBB* (OMIM*300481), maps at Xp21.1 and causes X-linked CGD (OMIM#306400), accounting for about 65–70% of cases. Its partner in the membrane, $p22^{phox}$, encoded by *CYBA* (OMIM*608508), maps at chromosome 16q24 and causes one of the three forms of autosomal recessive CGD (OMIM#233690), accounting for less than 5% of cases. The cytosolic factor $p47^{phox}$ is encoded by *NCF1* (OMIM*608512), located at 7q11.23, accounting for about 25% of cases (OMIM#233700). The other cytosolic factor, $p67^{phox}$, encoded by *NCF2* (OMIM*608515), is located at chromosome 1q25, and accounts for less than 5% of cases (OMIM#233710) [9, 142, 154, 155, 195]. The nomenclature for various levels of protein expression of $gp91^{phox}$ is somewhat confusing but informative: when $gp91^{phox}$ is absent, such as due to a stop codon or a deletion, it is referred to as $X91^0$; when reduced amounts of a hypofunctional protein are present, such as due to a splicing or promoter defect, $X91^-$; and when normal amounts of a nonfunctional protein is present, such as due to a missense mutation, $X91^+$ [165]. This nomenclature, while of interest scientifically, is probably much less useful for predicting clinical prognosis and assessment of infection risk than simply knowing the percent of normal control superoxide produced by the patient's neutrophils (see Sect. 4.7.4 for more details).

In general, X-linked CGD with first significant infection and diagnosis at an earlier age and more severe phenotype overall than $p47^{phox}$ deficiency [195]. As discussed in Sect. 4.5, a single case of a dominant negative mutation in *RAC-2* presented with an impaired neutrophil respiratory burst due to RAC's critical role in NADPH oxidase function, as well as impaired chemotaxis and adhesion, due to RAC's critical role in linking surface adhesion molecules to the cytoskeleton [95]. The rates of CGD cases appear about the same across all ethnic and racial groups though with very modest differences in the frequency of specific autosomal recessive forms of CGD, where in all cases about one third of the X-linked kindreds represent de novo mutations.

The X-linked carrier state for $gp91^{phox}$ is not entirely silent. Lyonization of the X chromosome leads to two populations of phagocytes in X-CGD carriers: one displays normal respiratory burst function, whereas the other population, which has inactivated the normal X chromosome and left the defective one active, has impaired respiratory burst activity. Therefore, X-CGD carriers give a characteristic mosaic pattern on respiratory burst testing of peripheral blood. Because of X-chromosome inactivation is thought to be a stochastic process occurring early in embryogenesis, there is a bell-shaped curve of variation in the distribution of female carriers with respect to the percent of CGD versus normal oxidase phenotype neutrophils in individual female carriers. The ratio of normal to CGD phenotype neutrophils in a female carrier is thought to be fixed for life, though some changes in skewing have been reported to occur in elderly female carriers. It is also of note that when an HLA-matched female carrier sister serves as a donor for HSCT of a male patient with X-linked CGD, if full donor chimerism occurs, the ratio of oxidase normal to CGD phenotype neutrophils that is observed in the transplant recipient exactly matches that seen in the donor sister. From these observations it is possible to conclude that as few as 5–10% of cells having normal respiratory burst activity is usually sufficient to prevent the occurrence of bacterial or fungal infections typical of CGD in the female carrier [83]. However, other manifestations of heterozygous carriage of X-linked CGD mutations include discoid lupus erythematosus-like lesions, aphthous ulcers, and photosensitivity [29, 93].

4.7.3 Clinical Manifestations

Infectious manifestations. CGD presents anywhere from infancy to late adulthood, but the majority of patients are diagnosed as toddlers and children. However, with improved diagnostic tools and increased knowledge among the general physician community about CGD, a growing number of patients with the $p47^{phox}$ form or mild variants of X-linked CGD are diagnosed in later childhood or adulthood [195].

The frequent sites of infection are lung, skin, lymph nodes, and liver. Osteomyelitis and perianal abscesses or fissures are also common [165, 195] (Table 4.4). Although gingivitis has been reported in CGD, surprisingly it is not a clinical problem in most patients with CGD. Pulmonary infection is typically pneumonia, but hilar lymphadenopathy, empyema, and lung abscesses also occur (Fig. 4.5). The microbiology of the infections in CGD is remarkable for

Table 4.4 Percentage prevalence of frequent infections by site in CGD patients

Type of infection	USA ($n = 368$) (%)	Japan ($n = 221$) (%)	Iran ($n = 41$) (%)	Germany ($n = 39$) (%)
Pneumonia	79	88	65	67
Abscess	68	77	53	41
Lymphadenitis	53	85	75	72
Osteomyelitis	25	22	21	15
Sepsis	18	28	ND	23
Reference	[195]	[80]	[120]	[100]

ND, Not determined

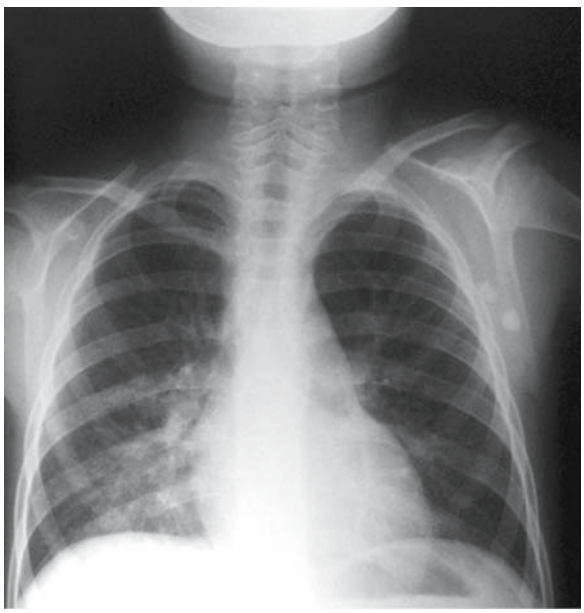

Fig. 4.5 Pneumonia and localized regional BCGitis in a 9-year-old X-linked CGD patient. Chest X-ray showing a right basal pneumonia and two calcified lymph nodes on the left axillae sequel to neonatal BCG vaccination

its relative specificity. The overwhelming majority of infections in CGD in North America are due to only five organisms: *Staphylococcus aureus, Burkholderia (Pseudomonas) cepacia, Serratia marcescens, Nocardia*, and *Aspergillus*. In the preprophylaxis era, most lung, skin, and bone infections were staphylococcal. Specific note must be made of the fact that particularly in infancy, but also in children under 2 years of age, osteomyelitis and soft tissue infection with *S. marcescens* is a particularly common first presentation of CGD. With TMP-SMX prophylaxis, the frequency of bacterial infections in general has been reduced. Staphylococcal infections in particular are essentially confined to the liver and lymph nodes [195]. Whereas the typical liver abscess in the immunocompetent patient involves enteric organisms and is liquid and easily drainable, the liver abscesses encountered in CGD are densely fibrotic caseous structures studded with microabscesses infected with staphylococcal and usually require excisional surgery for eradication. In some cases tube drainage or very prolonged medical therapy alone may suffice [104]. Bacteremia is uncommon, but when it occurs, is usually due to *B. cepacia, S. marcescens*, or *Chromobacterium violaceum*, one of the gram negative rods that inhabits soil and warm brackish water, such as that found in the south-eastern United States. Bacterial and *Nocardia* infections in CGD tend to be symptomatic and associated with elevated erythrocyte sedimentation rates, mildly elevated leukocyte counts, and fever [53]. In contrast, fungal infections are much less symptomatic in terms of leukocytosis or fever, and are often detected at asymptomatic stages. Unlike in neutropenic patients, fungal pneumonias do not generally cavitate in CGD, whereas *Nocardia* infections do. *S. marcescens* and *Nocardia* infections also tend to metastasize to multiple sites with a particular predilection for bone; and also tend to be necrotizing infections associated with extensive tissue destruction if not promptly and aggressively treated.

Fungal infections have been the leading cause of mortality in CGD [195]. However, the advent of itraconazole prophylaxis and the newer agents for treatment of filamentous fungal infections, such as voriconazole and posaconazole, have markedly reduced the frequency and mortality of fungal infections in CGD. Bony involvement by fungi typically but not exclusively occurs by direct extension from the lung to ribs or spine (Fig. 4.6). *Aspergillus nidulans* is an organism virtually exclusive in its occurrence in CGD. It causes a much higher rate of osteomyelitis than other fungi, and has had a much higher rate of mortality than *Aspergillus fumigatus* or other fungi [164, 179].

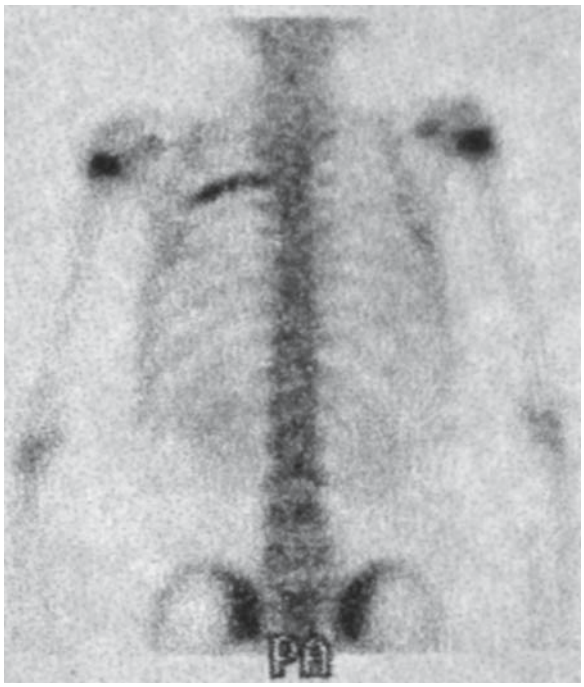

Fig. 4.6 Osteomyelitis in a 7-year-old X-linked CGD patient. Total body scintigram showing a posterior arch left rib fungal osteomyelitis in a CGD patient. His only manifestation was increased erythrocyte sedimentation rate in a routine follow-up laboratory control. No fever, pain or discomfort was reported at presentation or during follow-up

Besides *A. nidulans and C. violaceum*, other microorganisms should also encourage physicians to suspect CGD. *Granulibacter bethesdensis* is a novel gram-negative rod isolated from necrotizing lymph nodes in CGD [75]. *Penicillium piceum* is a relatively non-pathogenic fungus that produced lung nodules and osteomyelitis in a CGD patient [160].

Localized BCG infections are seen in CGD patients, although they rarely disseminate (Fig. 4.6). In the US CGD survey, almost 6% of pneumonias were reported to be mycobacterial [32, 195]. Rates of tuberculosis appear to be higher in CGD patients in some smaller series [96]. CGD patients appear to be more susceptible to tuberculosis and it is a significant problem for CGD patients in areas highly endemic for tuberculosis. The granulomas often seen in biopsies from CGD patients from any infectious or inflammatory may mislead pathologists to suggest tuberculosis despite the lack of confirming cultures or special stains. Therefore, a past history of tuberculosis in a CGD patient may or may not be accurate, and should be suspect in areas without significant endemic tuberculosis.

Non-infectious manifestations. Patients with CGD are prone to the formation of excessive granulomata (Table 4.5). These can affect any hollow viscera, but are especially problematic in the gastrointestinal (GI) and the genitourinary tracts (GU). Marciano et al. (2004), analyzed 140 CGD patients and found 43% of the X-linked and 11% of the autosomal recessive patients had symptomatic, biopsy–proven, inflammatory bowel disease. Abdominal pain was the most frequent symptom. Some children or adolescents without evidence of inflammation of the colon or sigmoid may have inflammation and granuloma formation confined only to the pylorus and/or upper bowel that may be difficult to diagnose by imaging or endoscopy. Symptoms may be only early satiety, discomfort after eating, occasional morning vomiting or even just vague, but chronic and generalized mild abdominal pain. The latter symptom complex when occurring in elementary school-aged CGD boys may be misinterpreted by parents and physicians as a psychosomatic symptom of school phobia. A trial of steroids should be considered before ascribing this symptom complex to psychosomatic causes in a young CGD patient. Walther et al. found 38% of patients had some kind of urologic event, including bladder granulomata, ureteral obstruction, and urinary tract infection. All patients with granulomata of the bladder or stricture of the ureter had defects of the membrane component of the NADPH oxidase: 8 had gp91^{phox} defects and 1 had a p22^{phox} defect [188]. Steroid therapy is quite effective and surprisingly well-tolerated for resolution of obstructive lesions or symptomatic granulomas of both the GI and GU tracts. In the case of sudden bladder obstruction with pain on urination in a child with known CGD who does not have evidence of urinary tract infection, instrumentation should specifically be avoided because prompt administration of a short course of high dose steroids may relieve the obstruction within 12–18 h. Several reports and many anecdotes confirm the benefit of steroids given at about 1 mg/kg for a brief initial period and

Table 4.5 Percentage prevalence of frequent granulomatous complications by site in CGD patients

Site of granulomatous complication	Percent of affected patients (patients in the study)	Reference
Gastrointestinal tract	32% (140)	[110]
Genitourinary tract	18% (60)	[188]
Choreoretinal lesions	24% (38)	[72]

then tapered to a low dose on alternate days [41, 121, 141, 178]. Prolonged low-dose maintenance (prednisone 0.1–0.2 mg/kd on alternate days) may seem to be only a homeopathic dose of steroids, but in many cases is both necessary and sufficient to control symptoms long term. Extensive experience with this level of very low dose alternate day prednisone has indicated that this doing does not appear to be associated with an increased rate of serious infections. There are anecdotal reports of the use of infliximab in severe cases of inflammatory bowel disease in CGD patients. While this therapy has been very effective in some patients who have significant fistulous tract formation or who are otherwise refractory to steroids or other agents, there is a very real risk of severe infections with typical CGD pathogens. Therefore, if infliximab is used for complicated or refractory inflammatory bowel disease in a CGD patient, intensified prophylaxis and increased vigilance for intercurrent infections are needed in the setting of this potent immunosuppressive.

Chorioretinal lesions are described in up to 24% of X-linked CGD patients. They are mostly asymptomatic retinal scars associated with pigment clumping. However, in a few cases large retinal inflammatory lesions develop with significant visual loss. While steroid treatment is not recommended for small quiescent lesions typically found in X-linked CGD patients. In cases of large active inflammatory lesions that may threaten vision, steroid treatment is often used, but there are no clear data to show that this intervention helps. Interestingly, these same lesions can also be detected in gp91^{phox} female carriers [72].

A peculiar and problematic inflammation complication of CGD occurring in the post-surgical patient is late dehiscence of surgical wounds without infection. This is particularly a problem in neck, chest and abdominal surgical wounds. The patient will have undergone surgery for diagnosis or treatment of an infection, but may also occur with hernia repair or other non-infection setting. Typically at about 7–12 days after the surgery (but can occur later), often after removal of clips or sutures, the surgical wound site will redden and show swelling. This will be followed by opening and spreading of the edges of the wound. If not properly managed the wound will take a very long time to heal by second intention granulation and late epithelialization leaving a very significant scar. Paradoxically, early intervention at the signs of early reddening and swelling of a surgical wound with 4–7 days of 0.5 mg/kg prednisone followed by a rapid taper over the next 2 weeks will prevent the dehiscence. Stitches or clips should be retained in place for a more prolonged period to prevent dehiscence in CGD patients.

Hepatic abnormalities are frequently described in CGD patients. Liver enzymes were reported to be elevated at least once in 73% of a CGD cohort (n = 194) followed at the National Institutes of Health (NIH), 25% had persistent elevations of alkaline phosphatase and drug-induced hepatitis was reported in at least 15% of these patients. One-half had splenomegaly that was usually associated with portal vein venopathy; in cases with abnormal liver enzymes who underwent biopsy liver histology, 75% had granulomata, and 90% had lobular hepatitis [84]. Development of significant venopathy with elevation of portal pressures in the liver together with significant non-immune mediated thrombocytopenia is a poor prognostic sign in CGD patients.

Autoimmune disorders of many types (generally of the Th1 variety) are more common in CGD. Both discoid and systemic lupus erythematosus (SLE) have been described in CGD patients, and also in X-linked CGD

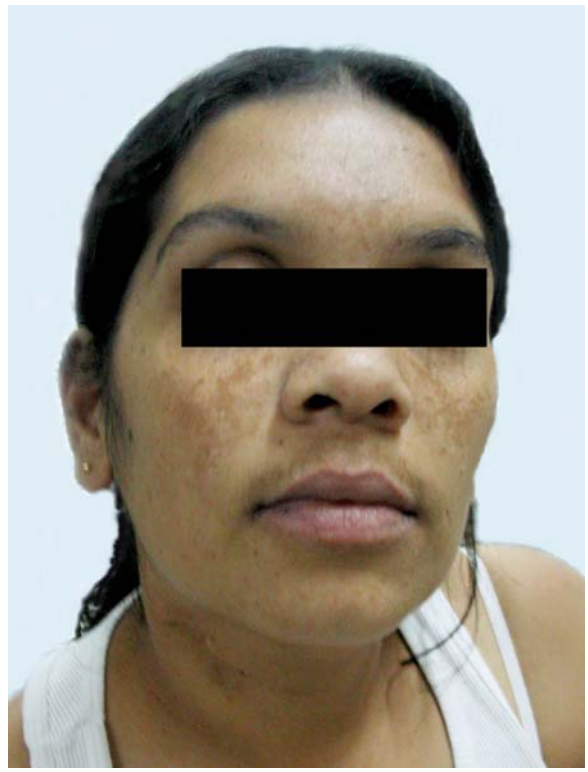

Fig. 4.7 Cutaneous manifestations in a female CGD carrier. Photosensitive discoid lupus-like lesions involving the cheeks of a 36-year-old X-linked CGD female carrier. A scar on the right side of her neck, secondary to lymphadenitis drainage, can also be seen

female carriers [34, 109] (Fig. 4.7). Sarcoidosis, idiopathic thrombocytopenic purpura (ITP) and juvenile idiopathic arthritis in children (previously called juvenile rheumatoid arthritis or JRA), antiphospholipid hypercoagulopathy, and idiopathic recurrent pericarditis all have been reported in children and young adults with CGD at a frequency higher than seen in the general population [195]. While many CGD patients have inflammation in the GI tract, a subset of these have a disorder indistinguishable from Crohn's disease. It is possible that Crohn's disease that occurs in CGD is part of the broad spectrum of autoimmune diseases that appear to be associated with CGD. The hyperinflammation of CGD and/or the recurrent infections may trigger the development of autoimmunity, which is influenced by predisposing genetic factors other than CGD.

The gene coding for the Kell blood cell antigen system (XK) maps to Xp21, immediately adjacent to *CYBB*, the gene for gp91^{phox}. Patients with interstitial deletions in the X-chromosome may delete portions of both genes (contiguous gene disorder) and thereby present with both CGD and McLeod syndrome, an acanthocytosis syndrome with anemia, elevated creatine phosphokinase, and late-onset peripheral and central nervous system manifestations. Special care has to be taken when transfusing X-linked CGD patients to avoid Kell(+) transfusions into Kell(-) patients [66, 115]. All X-linked CGD patients should be tested to be sure of the presence of Kell antigens.

Unlike many of the immunodeficiencies affecting lymphocytes, CGD patients are not more prone to develop neoplasia. Single cases of acute lymphoblastic leukemia and squamous cell carcinomata due to voriconazole photosensitivity are reported [116, 196].

4.7.4 Diagnosis

A history of recurrent and/or unusually severe infections, particularly those caused by the pathogens commonly associated with CGD (see 4.7.3 for more details), should prompt testing for this disorder. Although CGD has no pathognomonic clinical findings, the diagnosis should be particularly considered in the patient with a constellation of characteristic pathologies coupled with characteristic microbiology. Consistent clinical findings include splenomegaly, hepatomegaly, growth retardation, diarrhea, and abnormal wound healing with dehiscence, but these are neither necessary nor sufficient for the diagnosis. CGD patients may have minimal clinical signs and symptoms despite significant infectious involvement. Leukocyte counts are not consistently elevated during infection, whereas erythrocyte sedimentation rate is a sensitive indicator of infection. An increase in sedimentation rate or C-reactive protein should initiate the search for infection. Similar to other PID, diagnosis and treatment of infections in CGD must be aggressive. Invasive procedures oriented towards direct microbiological diagnoses should be considered as first-line diagnostic tests and should not be left until after the failure of empirical therapy. The reduction in mortality and morbidity in recent years is largely attributable to the prophylaxis of and aggressive recognition and treatment of infections in these patients [1, 68, 112, 173].

Diagnostic tests for CGD rely on various measures of superoxide production. These include direct measurement of superoxide production, ferricytochrome c reduction, chemiluminescence, nitroblue tetrazolium (NBT) reduction, and dihydrorhodamine-123 (DHR) oxidation. Currently, the flow cytometry-based DHR oxidation assay is preferred because of its objectivity, its relative ease of use, its ability to distinguish between X-linked and autosomal forms of CGD, and the ability to detect gp91^{phox} carriers [186, 187]. The other mentioned techniques are effective and can provide reliable diagnoses of CGD, but suffer either from an inability to distinguish individual cells, or the need for significant operator experience and interpretation.

Several other conditions may affect the neutrophil respiratory burst. Glucose-6-phosphate-dehydrogenase (G-6PD) deficiency, glutathione synthetase (GS) deficiency, and RAC-2 deficiency may mimic certain aspects of neutrophil dysfunction of CGD, such as the decreased respiratory burst and increased susceptibility to bacterial infections [152, 156, 192]. However, G-6PD deficiency is most often associated with some degree of hemolytic anemia, whereas CGD is not; on the other hand, severe GS deficiency is associated with 5-oxoprolinuria, acidosis and mental retardation, besides hemolytic anemia. Diverse pathogens, including *Legionella pneumophila, Toxoplasma gondii, Chlamydia, Entamoeba histolytica*, and *Ehrlichia risticii*, have been shown to inhibit the respiratory burst in vitro. Human granulocytic ehrlichiosis infection depresses the respiratory burst by downregulating gp91^{phox} [12].

Immunoblot and molecular sequencing are confirmatory techniques used to determine the specific genotype of CGD. Because gp91^{phox} and p22^{phox} require each other to stabilize their expression on the cell membrane, deficiency of either one of them usually implies the lack of the other in immunoblot analysis [165].

However, complete absence of any detectable signal in an "overdeveloped" anti-p22^{phox} blot together with lack of mosaic pattern of oxidase activity in maternal neutrophils is very strong presumptive evidence for genetic deficiency of p22^{phox}. The clinical history usually suggests autosomal recessive or X-linked disease, based on sex, consanguinity, age at presentation, and severity. Autosomal recessive p47^{phox}-deficient CGD has a significantly better prognosis than X-linked disease [195].

4.7.5 Management

The cornerstones of CGD management are: (1) early diagnosis, (2) Antimicrobial prophylaxis with TMP-SMX, itraconazole, and interferon-γ (IFNγ), and (3) aggressive management of infectious complications, which usually include invasive diagnostic procedures and parenteral/prolonged anti-infection medication. In this section, we will also discuss curative options for CGD.

Antimicrobial prophylaxis. CGD is the only primary immunodeficiency in which prospective, randomized, placebo-controlled studies of prophylaxis of infection have been performed [1, 68]. Anti-microbial prophylaxis in CGD patients relies on a triad of antibacterial prophylaxis [trimethoprim-sulfamethoxazole (TMP-SMX or cotrimoxazole)], antifungal prophylaxis (itraconazole) and immunostimulant therapy (IFNγ). Altogether this scheme has dramatically reduced the morbidity rates from severe infections [1, 68, 111, 112].

The first prophylactic agents shown to be effective in CGD patients were nafcillin and trimethoprim-sulfamethoxazole [90, 139]. With time, TMP-SMX became the standard of care for CGD patients. In a retrospective study, TMP-SMX (5 mg/kg/day) lowered the incidence of bacterial infections from 15.8 to 6.9/100 patient-months in X-linked CGD patients and from 7.1 to 2.4/100 patient-months in autosomal recessive CGD [112]. No increase in fungal infections was noted due to the use of TMP-SMX prophylaxis. TMP-SMX is preferred because it has activity toward the four most common bacterial pathogens causing infection in CGD patients (see Sect. 4.7.3 for more details), while at the same time having minimal effect on commensal bacterial flora in the gut.

Prophylactic TMP-SMX is usually prescribed at 5 mg/kg/day divided twice daily, although several centers use single-day doses to enhance treatment adherence. For patients allergic to sulfonamide drugs, alternatives include trimethoprim as a single agent, oral beta-lactamase stable penicillins such as dicloxacillin, an oral cephalosporin or a fluoroquinolone. In some young children, the antifolate effect of TMP-SMX may cause hematologic cytopenia, but in most cases may be corrected by the daily administration of leucovorin which bypasses the antifolate effect of TMP-SMX in human cells without blocking the antimicrobial effect of this antibiotic.

The advent of the azole antifungal drugs has dramatically altered the clinical consequences of fungal infections in CGD. Itraconazole is proven effective in CGD [33, 68, 119, 138]. In the only prospective, randomized, double-blind placebo-controlled antifungal trial in CGD, Gallin et al. reported 7 serious fungal infections in patients receiving placebo, compared to only 1 serious fungal infection in those receiving itraconazole (100 mg/day in patients aged 5–12 years; 200 mg/day in patients ≥13 years or ≥50 kg). The 39 patients in this study were randomized to receive placebo or itraconazole for a year and were then crossed-over to the other arm of the protocol; all patients were on antibacterial prophylaxis and most were receiving prophylactic IFNγ [68]. Itraconazole-resistant fungal infections do occur, but almost all have been responsive to voriconazole or posaconazole [4, 163]. The liquid oral formulation of itraconazole has significantly improved absorption than the capsule form but is slightly limited by the cyclodextrin vehicle, which poses some problems for the liquid formulation in renal failure.

Immunomodulatory therapy. An international, multi-center, randomized, double-blind, placebo-controlled trial, showed that IFNγ (50 mcg/m^2 subcutaneously three times weekly) reduces the number and severity of infections in CGD patients, regardless of their age, CGD genotype, or concomitant use of other prophylactic agents [1]. This study included 128 CGD patients (4–24 years old) from 13 centers (10 US, 3 European) and found the IFNγ was well tolerated. Marciano et al. confirmed the tolerability and long-term efficacy of IFNγ in a study involving 76 CGD patients followed for up to 9 years [111]. Based on 328 patient-years or observation, the incidence of serious infections on IFNγ was 0.30/patient-year, and the mortality rate was 1.5%/patient-year.

For patients over 0.5 m^2 of body surface, 50 mcg/m^2 three times weekly of IFNγ is recommended, while for children less than 0.5 m^2, 1.5 mcg/kg subcutaneously three times weekly is the suggested dose. Fever and myalgias are the most common IFNγ adverse events, but may be minimized by concomitant use of acetom-

inophen and administration before bedtime. Other side effects may include nightmares, general malaise and trouble concentrating on work tasks or school.

The need for administration by injection, cost, side effects, continuing improvement in prognosis based on better antifungals, and lack of general familiarity with cytokine therapies have contributed to the significantly less than universal use of IFNγ in CGD patients around the world [100, 119, 138]. It is possible that less frequent administration and/or lower doses of IFNγ might provide effective prophylaxis, but alternate regimens have not been studied. A number of patients find the standard dosing regimen unacceptable for a variety of reasons, but find lower doses and/or less frequent administration tolerable. Such alternate dosing likely provides benefit and is better than not taking IFNγ at all. Despite the strong evidence for IFNγ's prophylactic benefit in CGD, it has not been shown to help in the treatment of acute infections, nor does it either increase or decrease inflammation complications of CGD, including inflammatory bowel disease.

Acute infection management. Life-threatening infections may occur at any time in patients with CGD, even in those who have been free of infections for years. Serious infections, particularly those caused by fungi, may be asymptomatic or minimally symptomatic at presentation. Significant increases in sedimentation rate or C-reactive protein should prompt a search for hidden infection. Imaging with plain radiographs, ultrasound, CT, or MR imaging are extremely important for the detection of and determination of extent of infections. Because the differential diagnosis for any specific infection includes bacteria, *Nocardia*, mycobacteria, and fungi, a definitive microbiologic diagnosis is essential for directing therapy. Biopsies to obtain microbiological diagnosis should be insisted upon before the initiation of therapy and not after empirical therapy has failed (Fig. 4.8). Surgical debridement, especially in staphylococcal liver abscesses, may be necessary [104, 164]. In institutions which have experienced interventional radiologists, fine needle or core biopsies of lung or liver has a high yield for cytology or culture identification of causative pathogen (>70% yield) with less morbidity than open surgical procedures or even video-assisted thoracic surgery (VATS) lung biopsy. Having a cytology technician present at the time of needle biopsy can provide improve yield by providing a guide to whether a good specimen has been obtained (presence of

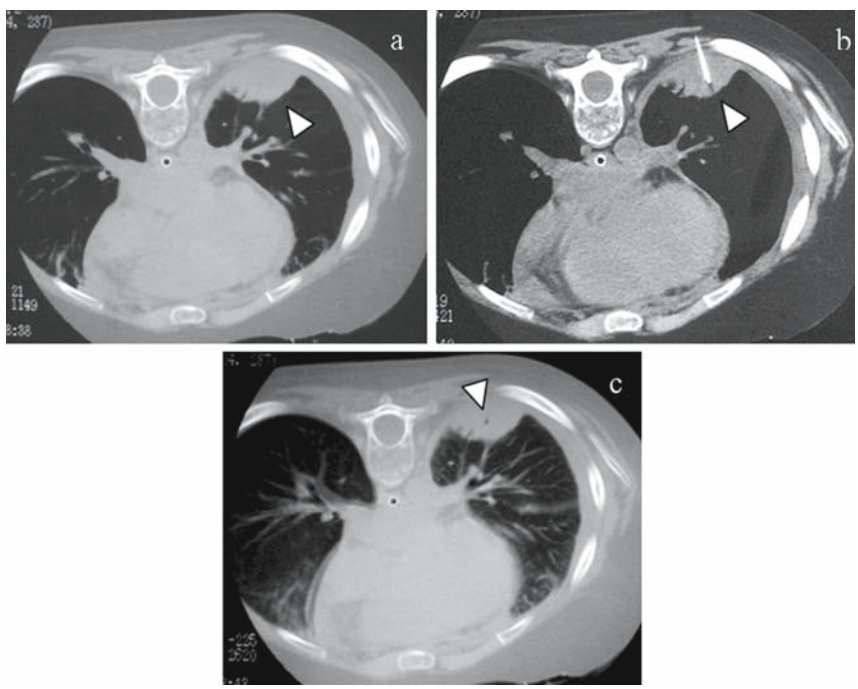

Fig. 4.8 Pre-, intra- and post-CT (computed tomography) scan-guided FNA (fine needle aspiration) in a 7-year-old X-linked CGD patient. (**a**) A thorax CT scan showing a pleural-based nodular lesion in the basal portion of the left lung (*white arrowhead*; the patient was placed on prone position for the procedure). (**b**) Pulmonary FNA biopsy performed with a 21G needle (*white arrowhead*). (**c**) Post- biopsy control CT scan where no complications are detected (e.g., bleeding, pneumothorax) and a small intralesional scar can be seen (*white arrowhead*)

granulomas) or if additional needle passes are required. Fungus infections of the lung in CGD generally have a low diagnostic yield by bronchoscopy, making biopsy a preferred diagnostic procedure.

Management of infections depends on the microbiology, but some general approaches can be outlined. For pneumonias, after diagnostic specimens have been obtained, empirical initiation of intravenous TMP-SMX plus either a fluoroquinolone or meropenem for bacteria/nocardia coverage, and voriconazole for fungus coverage is appropriate pending microbiology. Most *Burkholderia, Serratia* and *Nocardia* infections are sensitive to TMP-SMX. The use of TMP-SMX as therapy for infections that have occurred on prophylaxis remains highly effective, and may reflect either a dose-effect, or a failure of patients to actually take their prophylaxis. Staphylococcal pneumonias are very rare after the initiation of prophylaxis, but remain the most common cause of liver abscess, and may still cause lymph node infections. Lymphadenitis is usually staphylococcal and often necrotic. These infections may respond faster to excision of the affected lymph node along with antimicrobials. *Chromobacterium violaceum* is a Gram-negative rod that lives in warm brackish water and produces a deep purple pigment. It can cause bacteremia and sepsis in CGD, and typically responds to TMP-SMX, quinolones or carbapenems. *Granulibacter bethesdensis* is a newly-identified Gram-negative rod that causes necrotizing lymphadenitis in CGD. It grows slowly on *Legionella* or tuberculosis media and responds to ceftriaxone [75]. In general, fungal infections are more indolent and bacterial infections more acute in clinical presentation. However, Siddiqui et al. have recently described an acute fulminant pneumonitis with hypoxia due to inhalation of mulch or compost [172]. This presentation is sometimes the initial presentation of infection, even in an adolescent or young adult not previously known to have CGD, and is likely pathognomonic of CGD. The typical presentation is fever, cough and shortness of breath with hypoxia. Early in the process a regular chest X-ray may be read as normal, but computed tomography (CT) X-ray of the lungs reveals a diffuse reticular/miliary infiltrate. This must be considered a medical emergency because untreated, this type of infection progresses rapidly to fatal hypoxia because of an overwhelming pulmonary inflammatory response to the widely distributed inhaled fungus. Urgent institution of both antifungals and steroids (1 mg/kg/day) is required to treat the infection while reducing the potentially fatal severe pulmonary inflammation. As the infection comes under control the steroids may be reduced and tapered over several weeks.

Granulocyte transfusions have been used in severely ill CGD patients, especially those with fungal infections [85, 131, 185, 197]. However, with the remarkable improvement in antifungals over the last few years, the clinical reasons to use them are limited. Further, granulocyte transfusions often lead to alloimmunization, which may significantly impair the likelihood of successful HSCT. Therefore, we reserve granulocyte transfusions as a last resort if medical therapy is failing. If granulocytes transfusions are used, preparation of the donor by administration of a single dose of G-CSF plus dexamethasone the evening before apheresis collection results in extraordinarily large yields of granulocytes that when administered to an adult CGD patient may result in 5–20% circulating oxidase positive neutrophils at 12–15 h after administration.

Although HSCT is usually contraindicated in the setting of active infection, it has been used for refractory chronic infections in CGD. Ozsahin et al. controlled the infections and achieved full immune reconstitution in an eight-year-old boy with *Aspergillus nidulans* infection [131]. Bielorai et al. reported a similar case [23]. At this point, HSCT for active infections should only be performed at centers with experience in this procedure, as the risks of death are high.

Curative treatments. Successful HSCT is a definitive cure for CGD. In the largest series published, Seger et al. [166], reported very encouraging results in 27 mostly pediatric European CGD patients transplanted with unmodified marrow grafts from HLA-identical siblings (25/27) or unrelated (2/27) donors. Absence of pre-existing overt infection appeared as the single best prognostic factor for HSCT. All patients free of infections at the time of the transplantation (18/18) were well and alive at the time of publication. The 4 deaths in the study occurred among the 9 patients suffering from uncontrolled infections at the time of the procedure. The 4 cases of severe graft-versus-host disease described in their patients occurred in those with overt infections or acute inflammatory disease at the time of the transplant.

Since as few as 5–10% of normal cells are sufficient to prevent and control infections, as shown in Lyonized females, Horwitz et al. sought to achieve mixed hematopoietic chimerism sufficient to prevent infection in 10 patients with CGD [83]. They gave T cell depleted hematopoietic stem cell grafts from HLA-identical siblings. Three of the adult patients died 8–14 months after the initial procedure. Six of the 10 recipients had engraftment of at least 33%, but one had delayed graft

failure. The five recipients who survive and remain engrafted continue to be free of CGD infections or other complications of CGD at more than 7–9 years after transplantation. One of these patients has stable, unchanging mixed chimerism with 12% donor myeloid cell engraftment, indicating that even this modest percent of oxidase normal neutrophils is sufficient to achieve cure of the CGD phenotype.

Gungor et al. tested a shortened and less toxic conditioning protocol using bone marrow-derived stem cells in three high-risk adult CGD patients. Prior to the transplant these patients were also pretreated with intravenous antibiotics and antifungals. All survived the procedure with full donor engraftment and normal neutrophil function at 12–27 months [77].

These are promising preliminary results for HLA-identical HSCT in CGD patients. Still undefined are the proper degree of immune ablation in preparation for the transplant, the degree of T cell depletion, and the prophylaxis for graft versus host disease. The decision to pursue HSCT in CGD is an evolving one: as transplant-related morbidity and mortality decline, the benefits to patients in terms of rescue from inflammatory bowel disease, for instance, will be substantial. Currently, with best medical therapy, HSCT survival is high and complications are usually manageable; so, it may become 'the treatment of choice' in the foreseeable future [134].

Chronic granulomatous disease is also well-suited for gene therapy, since it results from single-gene defects that almost exclusively affect the hematopoietic system. Retroviral vectors that provide normal gp91^{phox}, p47^{phox} or p67^{phox} genes can reconstitute NADPH oxidase activity in deficient cells, establishing the proof-of-principle for gene therapy in CGD [51, 108, 190]. Malech et al. reported ex-vivo transduction of peripheral blood stem cells in 5 adult patients with p47^{phox} deficient CGD [108]. While functionally corrected granulocytes were detectable in peripheral blood following this procedure, their peak frequency was only 0.004–0.05 % of total peripheral granulocytes, a level well below the minimum number required for protective activity. Subsequently, Ott et al. reported two adults with X-linked CGD who were successfully treated with retrovirus-based gene therapy and autologous HSCT after busulfan-mediated non-ablative bone marrow conditioning. The substantial levels of oxidase positive neutrophils appeared to result from outgrowth of myeloid clones in which vector had inserted in and activated the EVI1/MDS gene complex or similar myeloid growth control genes with one or two clones predominating myelopoiesis. Clinical response was observed after transplantation in both patients. However, one of the patients died 27 months after the procedure due to infection and, at the time of death, gene marked neutrophils appeared to have lost oxidase activity. The second patient also appeared to have diminished oxidase activity over time despite continued gene marking. The long-term consequences of vector insertion mediated clonal outgrowth and oligoclonality of myelogenesis is unknown, but myelodysplasia may be a risk. The long-term risks and effectiveness of gene therapy for CGD remain to be determined [129, 130].

Prognosis. When the first 92 patients with "fatal granulomatosis of childhood" were reported, 45 had already died, 34 of them before the age of 7 years. Today, survival is dramatically improved. In the United States CGD registry, more than 25% of all living CGD patients (and 42% of those with autosomal recessive CGD) were 20 years or older [195]. In a German cohort of 39 patients observed over a 22-year period, the survival rate was 50% through the fourth decade of life [100]. In a British cohort, aggressive antibacterial and antifungal prophylaxis greatly diminished the risk of serious infections compared with historic controls [33].

The quantity and quality of the lives of CGD patients have improved dramatically since its initial description. Life-threatening infections continue to occur, but diagnostic and treatment opportunities have improved as well, making CGD a disease that is eminently survivable. Focus on improved management approaches for the significant complications of CGD, such as inflammatory bowel disease and chronic hypoxic inflammatory/fibrotic lung disease, is sorely needed. HSCT and gene therapy are improving and eventually will offer definitive correction. In the interim, antimicrobial prophylaxis with TMP-SMX, itraconazole and IFNγ; early diagnosis of infections and aggressive treatment of them; and aggressive management of CGD-associated colitis and inflammatory/fibrotic lung disease will keep patients well.

4.8 Neutrophil G-6PD Deficiency

4.8.1 Definition

Glucose-6-phosphate-dehydrogenase (G-6PD) deficiency (OMIM+305900) is one of the most common enzymopathies in erythrocytes, which causes usually a nonspherocytic anemia without immunologic abnormalities. In 1972, Cooper et al. described a white female patient with severe bacterial infections and absence of

G6PD in leukocytes. It was demonstrated that the patients' leukocytes were unable to kill ingested bacteria [44].

4.8.2 Etiology

G-6PD deficiency is due to missense or small in-frame mutations in the *G6PD* gene [22] on Xq28. The reduced function of the enzyme in leukocytes leads to a reduction of NADPH by the hexose-monophosphate pathway. The most susceptible cells are those that lack mitochondria-like erythrocytes because these cells need NADPH against oxidative stress and have no other source of this molecule than the hexose-monophosphate pathway. If the mutation is severe enough that the residual activity is below 5% [11], the oxidative burst of phagocytes is also severely impaired [44, 74, 184].

4.8.3 Clinical Manifestations

Severe phenotypic forms present with symptoms similar to CGD, like organ abscesses, severe pneumonia and signs of hemolytic anemia (jaundice, elevated reticulocyte count) [74].

G-6PD deficiency is highly polymorphic and the severity of clinical manifestations depends on the variant alleles: the most frequent variants are the Mediterranean and Canton variants, which present as severe phenotype, whilst the African and Mahidol variants present as moderate disease phenotype [128]. Interestingly, CGD-like forms are only observed in Caucasians with G-6PD deficiency [127].

4.8.4 Diagnosis

Signs of hemolytic anemia in combination with a 'pathological' nitro blue-tetrazolium (NBT) test or dihydrorhodamine reduction below 1% suggest the diagnosis. Determination of G-6PD activity in erythrocytes and leukocytes using a fluorescent spot test [21] or spectrophotometrically measured NADPH generation [20] confirms the diagnosis.

4.8.5 Management

There is no curative treatment available. Acute infections and hemolysis have to be treated symptomatically.

4.9 Myeloperoxidase Deficiency

4.9.1 Definition

Myeloperoxidase (MPO) deficiency (OMIM#254600) is the most common phagocytes defects (approximately 1 in 4,000 population) and leads to a defective production of hypochloric acid in these cells [122, 133]. It was first described by Lehrer and Cline [98], who found no detectable activity of the lysosomal enzyme in neutrophils and monocytes from a patient with disseminated candidiasis. Other granule-associated enzymes were normal. Leukocytes from one of the proband's sisters also showed no MPO activity. Leukocytes from the proband's four sons showed about one-third normal levels. Salmon et al. [159] demonstrated immunologically the absence of MPO protein, or at least the absence of cross-reacting material in homozygotes. Eosinophil peroxidase, which is chemically distinct from MPO, was normal.

4.9.2 Etiology

Myeloperoxidase is abundant in azurophilic granules and catalyzes the conversion of H_2O_2 into hypochloric acid [123]. This molecule amplifies the toxicity of reactive oxygen radicals. The gene is encoded on chromosom 17q23. The primary deficiency of MPO is inherited as an autosomal recessive disorder. A secondary form of MPO deficiency has been described in lead poisoning (due to inhibition of heme synthesis), in severe infections (due to consumption), neuronal lipofuscinosis, diabetes mellitus, in patients treated with cytotoxic drugs and malignant disorders like acute and chronic myeloid leukemia, myelodysplastic syndrome and Hodgkin's lymphoma (due to chromosomal rearrangements). MPO-deficient neutrophils are markedly less efficient in killing *Candida albicans* or *Aspergillus hyphae*.

4.9.3 Clinical Manifestations

Interestingly, the vast majority (>95%) of MPO deficient individuals are completely asymptomatic, despite the killing defect of the neutrophils. Symptomatic patients suffer from recurrent candida infections and sometimes from

diabetes mellitus [39, 133]. Severe infections of the bones, meninges and septic episodes occasionally occur.

Anti-MPO antibodies are associated with certain forms of vasculitis (e.g., microscopic polyangiitis) and MPO derived oxidants seem to play a role in neurodegenerative disorders and atherosclerosis [123, 135, 183], but this is not uniformly accepted [117]. Interestingly, MPO knockout mice developed larger atherogenic lesions under a high cholesterol diet than MPO wild type mice [30].

4.9.4 Diagnosis

MPO deficiency can be suspected if a large proportion of "unstained" cells are reported from a differential blood count. The definite diagnosis requires the demonstration of the defect enzyme. MPO is easily detected using a hydrogen peroxide/ethanol solution containing benzidine. Cells with intact enzyme show yellow-brown granules in the plasma, cells with MPO deficiency have clear plasma around the blue cell nucleus.

4.9.5 Management

There is no specific treatment for MPO deficiency. In symptomatic patients long-term antifungal prophylaxis with fluconazole or itraconazole may be beneficial. In patients with diabetes mellitus rigorous control of the blood glucose should be achieved.

4.10 Specific Granule Deficiency

4.10.1 Definition

Specific granule deficiency (SGD) (OMIM#245480) is a very rare deficiency of neutrophil granules which leads to disturbed chemotaxis and receptor upregulation and increased susceptibility to bacterial infections (Fig. 4.9).

4.10.2 Etiology

The granulocytes lack expression of at least one primary and all secondary and tertiary granule proteins. The lack of many granule constituents results in a significant decrease of oxygen-independent bactericidal activity and a decrease in expression of adhesion molecules and chemotactic receptors on the cell surface.

The defect is caused by a mutation in a myelopoiesis specific transcription factor (*CEBPε*) or Ccaat/enhancer-binding protein, epsilon (OMIM*600749) [99], which regulates the synthesis of proteins at the critical period during differentiation of neutrophils that encompasses the tail end of primary granule production and all of the period of time during which the specific granules and it components are produced. In addition to granule contents, the membranes of these missing granules would normally contain receptors for chemotactic factors like fLMP or adhesion proteins. Specific granule deficiency is an oxygen independent microbicidal defect. Targeted disruption of the gene in mice resulted in a phenotype very similar to humans. This includes bilobed nuclei, abnormal respiratory burst activity, and impaired chemotaxis and bactericidal activity. The CEBPε-deficient mice are susceptible to gram negative bacterial sepsis, particularly with *Pseudomonas aeruginosa*, and succumb to systemic infection at 3–5 months of age [73].

4.10.3 Clinical Manifestations

The patients suffer from ulcerative and necrotic lesions of the skin and mucous membranes as well as recurrent pneumonias frequently due to *Staphylococcus aureus* and/or *Pseudomonas aeruginosa*. As in LAD, pus formation is defective.

4.10.4 Diagnosis

In the blood smear, abnormal segmentations of the granulocytes are pathognomonic. Chemotaxis is significantly reduced and specific granules are absent from electron microscope images of granulocytes. As SGD individuals express normal levels of lactoferrin and transcobalamin I in their saliva but not in their plasma or neutrophils, determination of these two molecules in the two compartments may give a hint for the diagnosis. Definitive diagnosis is made by mutational analysis of the *CEBPε* gene.

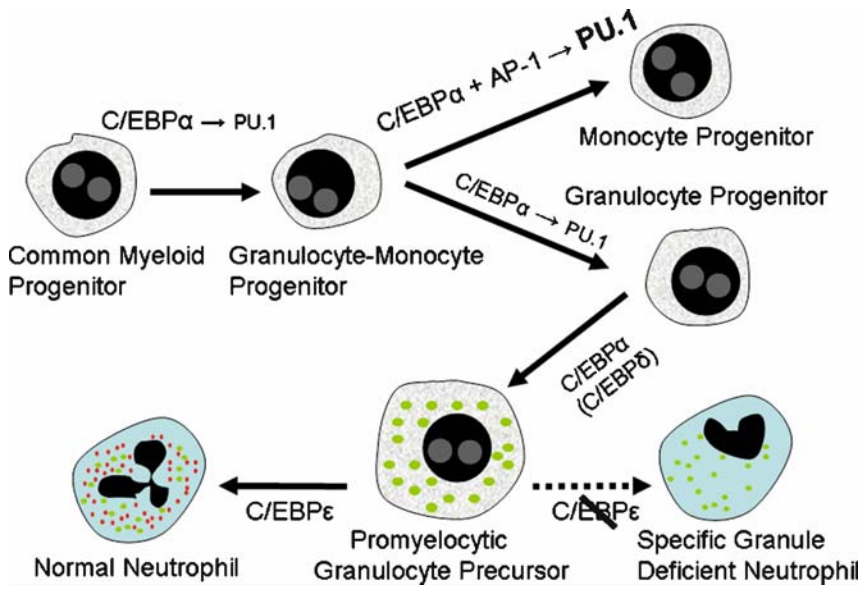

Fig. 4.9 Some of the members of the CCAAT/enhancer binding protein (C/EBP) family of DNA regulatory molecules play key roles in the development and differentiation of myeloid cells. This figure indicates the particularly essential role of C/EBPα and C/EBPε in granulopoiesis with emphasis on where in differentiation of neutrophils loss of function mutations of C/EBPε leads to specific granule deficiency phenotype. Growth factors and differentiation signals impinging on the common myeloid progenitor that enhance the production of C/EBPα lead to modest production of PU.1, another DNA regulatory factor that drives differentiation toward the granulocyte-monocyte progenitor. Growth signals conducive to monocyte differentiation mediate their effect by inducing production of AP-1 and other regulatory molecules which result in high levels of PU.1 that drive differentiation toward monocytogenesis. Interestingly, loss of C/EBPα blocks production of neutrophils and eosinophils, but does not fully block monocyte production. Growth signals conducive to granulocyte differentiation mediate their effect by maintaining C/EBPα, but with a low level of PU.1, driving differentiation toward the promyelocytic stage of differentiation. There is some evidence that C/EBPδ may play an important permissive role at this stage of granulopoiesis. At the late stage promyelocyte in the last phase of production of azurophil granules C/EBPε is absolutely required for activation and transcription of genes encoding some proteins that are packaged in the last group of azurophil granules, for all the proteins packaged in specific granules, for proteins needed to construct the actual specific granule structures, and for proteins required for producing the characteristic nuclear segmentation of mature neutrophils. Thus, in the absence of functional C/EBPε neutrophils are produced but lack some azurophil granule proteins, lack all specific granule proteins, and have incomplete neutrophil nuclear segmentation (*lower right side* of figure)

4.10.5 Management

Long-term antibiotic prophylaxis is usually necessary. Antibiotics in acute infections should cover *Staphyloccus aureus, Pseudomonas aeruginosa* and *Klebsiella* spp.

4.11 Shwachman–Diamond Syndrome

4.11.1 Definition

Shwachman–Diamond syndrome (SDS) (OMIM #260400) is a syndrome comprising exocrine pancreatic insufficiency, bone marrow failure and metaphyseal chondrodysplasia. It was first described by Bodian et al. in 1964 [24] and subsequently by Shwachman, Diamond et al. in the same year [171]. It affects approximately 1 in 50,000 live births. In 2003, mutations in the *SBDS* gene (Shwachman–Bodian–Diamond syndrome) were found to be associated with the clinical disease [27].

4.11.2 Etiology

SDS is a disease caused by mutations in a hitherto poorly characterized gene called Shwachman–Bodian–Diamond-Syndrome gene (*SBDS*) (OMIM*607444). Most *SBDS* mutations appear to arise from a gene con-

version event between the *SBDS* gene and its adjacent pseudogene [27]. *SBDS* co-precipitate with molecules like 28S rRNA and nucleofosmin. The latter protein is implicated in the regulation of ribosome biogenesis [76], modulation of apoptosis [132] and chromatin transcription [180]. Homozygous expression of *SBDS* gene mutations leads to early fatal death, suggesting that the *SBDS* gene is essential for early mammalian development [202]. There is therefore some experimental support that SDS belongs to bone marrow failure syndromes affecting the ribosome [69] like dyskeratosis congenita [118] or Blackfan-Diamond anemia [42]. The syndrome encompasses a moderate neutropenia with exocrine pancreatic insufficiency and metaphyseal chondrodysplasia. Later on, anemia and thrombocytopenia develop in a majority of patients. Neutrophils show defective chemotaxis [2]. The amount of CD34 cells is reduced and the CD34 cells have a reduced capacity to form colonies. Apoptosis of CD34 cells is increased [54–57] which may explain the pancytopenia.

4.11.3 Clinical Manifestations

Patients with SDS suffer as infants initially from failure to thrive with foul smelling stools due to the pancreatic insufficiency and persistent or intermittent neutropenia with recurrent infections like recurrent otitis media, sepsis, pneumonia etc. [71]. Later on pancreatic insufficiency improves significantly in more than 50% of the patients older than 4 years, but anemia as well as thrombocytopenia develops in a high proportion of patients (up to 40%). Neutropenia is intermittent in about two-thirds and constant in the remaining third [71]. Approximately 10% of patients progress to myelodysplastic syndrome and acute myelogenous leukemia [176]. Furthermore, patients suffer from skeletal abnormalities (irregularity of metaphyses, osteopenia, short stature) [106], neurodevelopmental delay [87], dental caries [2], and hepatic dysfunction [71].

4.11.4 Diagnosis

A presumptive diagnosis requires the demonstration of exocrine pancreatic insufficiency (increased fat in stool sample) and bone marrow failure, i.e. neutropenia (<1,500/μl, 3 times over 3 months), thrombocytopenia (<150,000/μl), and anemia. Abdominal ultrasound shows typically an echointense pancreas (Fig. 4.10) due to replacement of acini with adipose tissue (Fig. 4.11). Chemotaxis of neutrophils is reduced and some patients show a metaphyseal dysplasia on long bone radiology. The diagnosis could be confirmed by mutational analysis of the *SBDS* gene, but a negative test does not exclude the diagnosis, as about 10% of patients with a clinical diagnosis of SDS lack *SBDS* mutations. In the patients younger than 3 years, serum trypsinogen is pathologically low.

Laboratory tests should include a complete blood and differential count, 72-h fecal fat collection, serum trypsinogen, bone marrow aspiration with cytogenetic studies particularly to look for myelodysplastic syndromes (MDS) and monosomy 7, imaging of the

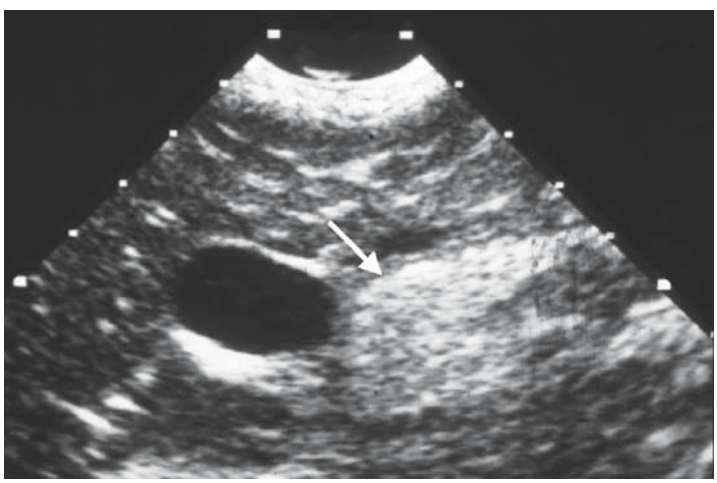

Fig. 4.10 Abdominal sonography of a 2-year-old boy with SDS and typical "white" pancreas (*arrows*) due to lipomatosis. (Courtesy of K. Schneider; Munich, Germany)

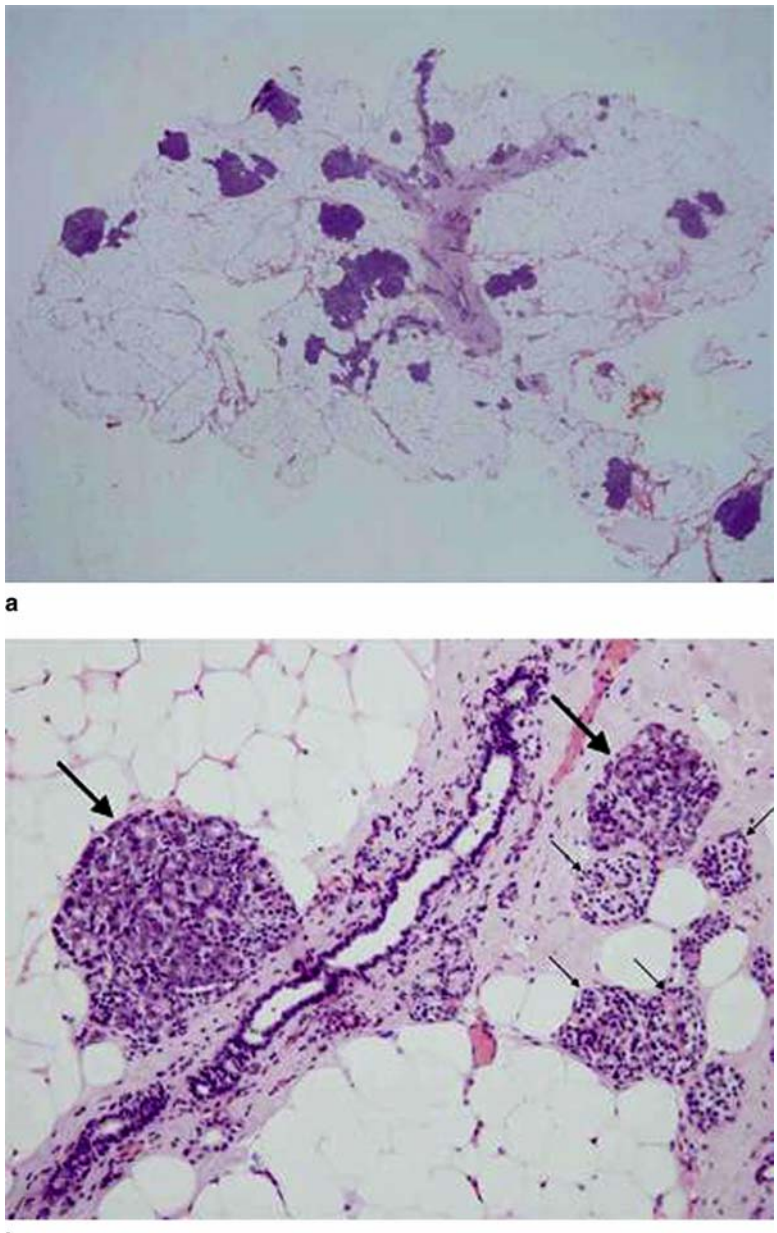

Fig. 4.11 Typical histology of the pancreas of a patient with SDS. Note the extensive replacement of the exocrine pancreas by adipose tissue surrounding acini (*large arrows*) with remaining small islands of parenchyma (*small arrows*). (**a** and **b** different magnifications)

pancreas, and long bone radiology. Cystic fibrosis should be excluded.

4.11.5 Management

First line therapy is directed to ameliorate the direct consequences of the disease. Exocrine pancreatic failure is treated with substitution of pancreatic enzymes similar to cystic fibrosis and fat soluble vitamins if needed. Blood count should be monitored at least every 6 months and bone marrow once a year. Neutropenia with recurrent bacterial infections or with a high risk of severe infections (e.g., ANC <500/μl) can be treated with G-CSF. There is, however, a theoretical risk of stimulation of malignant pre-leukemic clones and therefore the risks and benefits should be considered. Leukocyte-depleted and irradiated erythrocyte transfusions are recommended in patients with symptomatic anemia. In cases of thrombocytopenia and bleeding platelet, transfusions are indicated. HSCT should be offered to

patients with pancytopenia, MDS or overt leukemia in remission [40, 169, 170]. HSCT may be complicated by the stromal defect and should be performed in centers with experience with this disease. Survival is only about 60–70%. Finally, for bone and dental abnormalities anticipatory management is indicated.

4.12 Localised Juvenile Periodontitis

4.12.1 Definition

Localised juvenile (prepubertal) periodontitis (OMIM#170650) is a form of aggressive periodontitis that occurs in the primary dentition of children. In the absence of systemic disease it is thought to be a special form of the more frequently occurring localized aggressive periodontitis in adolescences and adults. Neutrophils show impaired chemotaxis.

4.12.2 Etiology

The disease is thought to be caused by the absence of a cell surface glycoprotein (GP110) [28, 136]. The exact pathogenic mechanism, however, is not known. It has been suggested that mutations in a chemotactic receptor, formyl peptide receptor 1 (*FRP1*, OMIM*136537) could lead to such disease [78].

4.12.3 Clinical Manifestations

The disease is characterized by symmetric localized loss of attachment of primary teeth, (Fig. 4.12) gingival inflammation, extensive plaque deposits and calculus. It may progress to localized aggressive periodontitis in the permanent dentition. *Actinobacillus actinomycetum* species are frequently isolated from gingival swabs.

4.12.4 Diagnosis

Inspection of the oral cavity with typical clinical signs, impaired chemotaxis to fMLP [167] and lack of systemic disease. Definitive diagnosis can be achieved by mutational analysis of the chemotactic receptor *FPR1*.

4.12.5 Management

Therapy includes regular local cleaning and antibiotic therapy to reduce plaque formation and extraction of affected teeth. Combination therapy with amoxicillin and metronidazole seems to be particularly effective [161, 168]. Nevertheless, periodontal surgery is often necessary.

4.13 Papillon–Lefèvre Syndrome

4.13.1 Definition

Papillon-Lefèvre syndrome (OMIM#245000) is characterized by premature loss of the primary and permanent teeth, hyperkeratosis of the palms, soles and less frequently knees and elbows.

4.13.2 Etiology

The gene responsible for this disease is the cathepsin C gene (*CTSC*) (OMIM*602365), located on chromosome

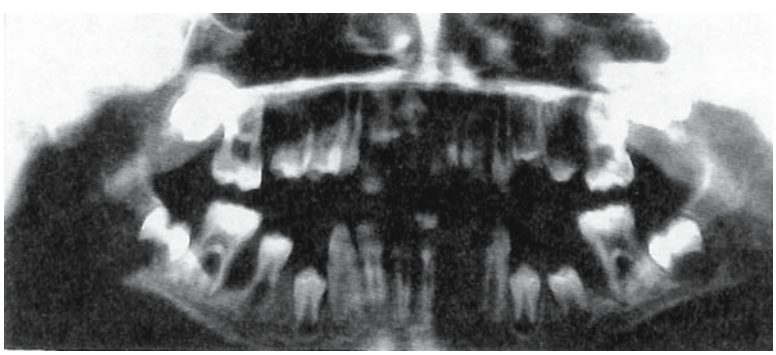

Fig. 4.12 Horizontal resorption of alveolar bone in a patient with localized juvenile periodontitis. (Courtesy of B.H. Belohradsky; Munich, Germany)

11Q14 [128]. This leads to a defective function of the neutrophils [64], which alleviate the gingival infection. *Actinobacillus actinomycetenicomitans* species, *Fusobacterium nudatum, Eikenella corrodens* are typical bacteria cultured from the gingival sulcus [193]. The loss of the teeth is a consequence of the gingival inflammation.

4.13.3 Clinical Manifestations

Typical symptoms are periodontal inflammation soon after eruption of the primary teeth with rapid and severe bone loss; in general primary teeth are lost by 5 years and permanent teeth a few years after eruption.

4.13.4 Diagnosis

Inspection of the oral cavity with typical clinical signs, and hyperkeratosis of the palms, soles, knees, and elbows associated with impaired chemotaxis. Definitive diagnosis can be achieved by mutational analysis of the *CTSC* gene.

4.13.5 Management

Early antibiotic therapy specific for the above mentioned pathogens normally slow the development of the disease. If antibiotics fail, extraction of all erupted teeth should be performed to preserve the non erupted permanent teeth. Treatment with retinoids has been reported with variable success [94, 182].

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