bjh review

Recent advances in the understanding of genetic defects of neutrophil number and function

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Summary

Neutrophils are amongst the first immune cells to arrive at sites of infection and play an important role as the host's first line of defence against invading pathogens. Defects of neutrophil number or function are usually recognized clinically by recurrent infections that often are life-threatening. Over the last few years, a number of genetic mutations have been discovered to be the basis for congenital neutropenia, adding to our understanding of the molecular basis of these diseases. While many genetic mutations that cause severe congenital neutropenia result in a differentiation block at the promyelocyte stage, defects of neutrophil function are more heterogeneous on clinical, genetic and mechanistic levels. In this review we discuss recent advances in our understanding of the genetic and molecular basis of human neutrophil disorders.

Keywords: neutrophils, neutropenia, neutrophil function, immunodeficiency, oxidative burst, adhesion, cell migration.

Polymorphonuclear neutrophils are the most abundant leucocytes in the blood and form the first line of host defence. Within the bone marrow, they differentiate from multipotent myeloid stem cells through several generations of morphologically recognizable cells. In order of maturation these are, the myeloblast, promyelocyte, myelocyte, metamyelocyte, band neutrophil and segmented neutrophil (see Fig 1). After the myelocyte stage, the capacity for significant cell division is lost and maturation from myeloblast to mature segmented neutrophil takes 10–12 d. Mature neutrophils then leave the bone marrow and enter the circulation. The average half life of neutrophils in the blood is about 7 h before they extravasate into peripheral tissues. During an infection and the subsequent inflammatory reaction, neutrophil production and activation is initiated by enhanced proliferation and maturation of precursor cells in the bone marrow, which are then released into the circulation at an accelerated rate (Nathan, 2006). Circulating neutrophils express specific adhesive receptors, which aid in rolling, tethering and firm adhesion to the vascular endothelium that preceeds neutrophil extravasation (see Fig 2). During inflammation, gradients of chemokinetic and chemotactic molecules direct the neutrophils to traverse the vascular endothelial wall and migrate into peripheral tissue towards the site of infection. Interactions between the neutrophil and microbe induce phagocytosis, degranulation and activation of the respiratory burst, which in combination mediate microbial killing primarily within the phagocytic vacuole (see Fig 2) (Nathan, 2006). Microbial killing is further enhanced by formation of neutrophil extracellular traps (NETs), which are fibrous networks composed of chromatin, DNA and proteins from antimicrobial granules, formed by activated neutrophils to assist in killing and prevention of spreading of bacteria (Brinkmann *et al*, 2004). Defects in any part of this complex process manifest as immunodeficiency of neutrophil function with life-threatening infections. Here we describe recent insights into the genetic defects and mechanisms underlying primary neutrophil defects.

Defects of neutrophil differentiation

Severe congenital neutropenia and cyclic neutropenia

Severe congenital neutropenia (SCN) is a heterogeneous disease that results from mutations in a variety of genes (Table I). Although generally seen as a monogenic disorder, a recent report has identified four SCN patients with mutations in multiple genes that are found as monogenic cause for SCN. While it is predicted that in these patients one genetic mutation is dominant disease causative, a second mutation could have a synergistic effect and worsen disease phenotype (Germeshausen *et al*, 2010).

Neutrophil formation in SCN patients is arrested at the promyelocytic stage in bone marrow (Fig 1). Patients are usually diagnosed during the first year of life with very low circulating neutrophil counts, resulting in life-threatening infections (Welte *et al*, 2006). In contrast, patients with cyclic neutropenia usually present later in life and show regular and consistent oscillations (with a mean of 21-d cycles) of their number of circulating neutrophils and have a milder clinical course. At the nadir of the

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First published online 31 August 2010
doi:10.1111/j.1365-2141.2010.08361.x

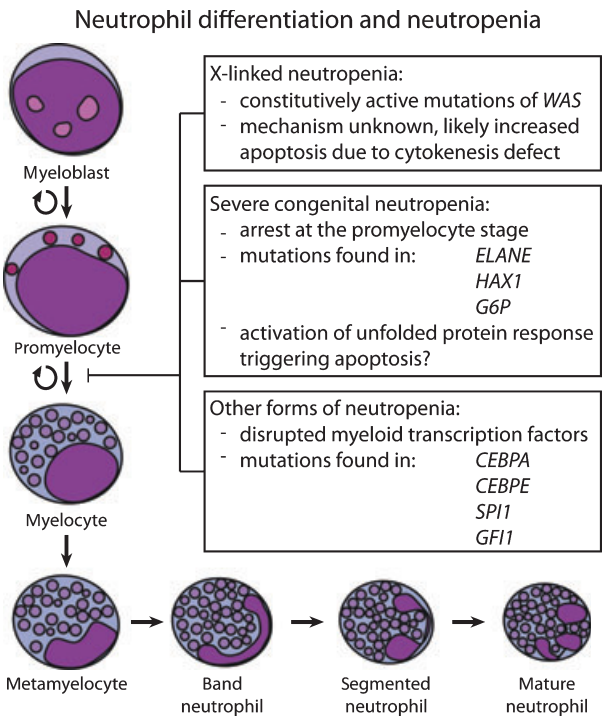

Fig 1. Schematic overview of the stages of neutrophil differentiation. Different forms of neutropenia are shown in the boxes and the associated genes or transcription factors indicated. The majority of congenital neutropenias result in arrest of neutrophil differentiation at the promyelocyte stage. A similar arrest has been reported for some, but not all patients with X-linked neutropenia and other mechanisms may contribute to neutropenia in this disorder. Straight arrows indicate progressive maturation and circular arrows denote stages at which cells division can occur.

neutrophil count, which lasts from 3 to 6 d, patients suffer from fever, mouth ulcers and infections (Dale & Hammond, 1988; Haurie *et al*, 1998). Over 90% of patients with either disorder respond to granulocyte colony-stimulating factor (G-CSF) therapy (Dale *et al*, 1993; Rosenberg *et al*, 2006). In refractory cases, or where SCN is complicated by haematological malignancy, haematopoietic stem cell transplantation is a potentially curative option. Data from the Severe Chronic Neutropenia International Registry (http://depts.washington.edu/registry/) indicates a significantly higher than previously recognized cumulative incidence (21%) of developing acute myeloid leukaemia (AML) or myelodysplasic syndrome (MDS) (Freedman & Alter, 2002; Dale *et al*, 2006; Rosenberg *et al*, 2006, 2010). Long term follow up from this registry revealed that the risk of developing AML/MDS was significantly lower than initially thought (2·3%/year compared to 4–12%/year), although the cumulative incidence stays high (22%) after 15 years of follow-up (Rosenberg *et al*, 2010). It has now been demonstrated that the highest risk of AML/MDS is in the cohort of patients that needs greater than the median dose of G-CSF but has less than the median neutrophil response. The most likely explanation of this observation is that G-CSF merely allows those children with the severest disease to live long enough for the full phenotype to manifest, but it is clearly impossible to totally exclude a leukemogenic effect of exogenous G-CSF. Mutations in the G-CSF receptor are associated with progression in some cases, but may persist for many years without progression and are not an absolute requirement for the development of leukaemia, proving a causative association is confounded by the fact that almost all patients are on G-CSF treatment (Dong *et al*, 1995; Tidow *et al*, 1997; Ancliff *et al*, 2003; Cassinat *et al*, 2004; Rosenberg *et al*, 2006, 2010; Zeidler *et al*, 2009).

ELANE

Several genetic lesions have recently been identified that account for a substantial proportion of cases of SCN or cyclic neutropenia. Mutations in the *ELANE* (previously *ELA2*) gene, encoding neutrophil elastase, were first identified as a cause for cyclic neutropenia (Horwitz *et al*, 1999) and heterozygous *ELANE* mutations were subsequently found to account for around 50% of autosomal dominant SCN cases (Horwitz *et al*, 1999; Dale *et al*, 2000; Ancliff *et al*, 2001; Bellanne-Chantelot *et al*, 2004; Klein, 2009; Xia *et al*, 2009; Zeidler *et al*, 2009). At least 52 different mutations in *ELANE* have been described, including mutations in prodomains and promoter region. The diversity of mutations in SCN appears wider than in cyclic neutropenia, but there is no clear relationship between particular *ELANE* mutations and the clinical form of disease with the exception of the G185R mutation. The G185R mutation introduces a positively charged arginine residue in the immediate proximity of the active site of neutrophil elastase and is likely to alter its biological function. Patients with this mutation appear to have very severe SCN with neutrophil counts close to zero, are refractory to G-CSF or require high G-CSF doses and are more prone to develop leukaemia (Dale *et al*, 2000; Bellanne-Chantelot *et al*, 2004; Horwitz *et al*, 2007).

Neutrophil elastase is a monomeric, 218-amino acid (25 kDa), chymotryptic serine protease, which is synthesized during the early stages of primary granule production in promyelocytes. It is formed as a proenzyme during neutrophil differentiation and stored in azurophilic granules in its active form, ready for release from the granules with full enzymatic activity at sites of inflammation (Bode *et al*, 1989). The mechanism by which elastase defects result in neutropenia remains under investigation. Loss of function is not the primary mechanism as no consistent defects of proteolytic activity, substrate specificity or serpin inhibition were found for elastase mutants (Li & Horwitz, 2001). Furthermore, genetically targeted elastase null mutant mice are not neutropenic (Belaaouaj *et al*, 1998), nor more interestingly, were mice targeted with a disease-causing *ELANE* mutant (Grenda *et al*, 2002). Two studies have suggested a role for *ELANE* mutations in activation of the unfolded protein response (UPR) and apoptosis in SCN pathogenesis. The UPR is triggered by accumulation of misfolded proteins in the endoplasmic reticulum (ER) and may lead to apoptosis of the cell. UPR involves three major mechanisms: attenuation of global protein synthe-

Table I. Disorders of neutrophil differentiation.

Disease	Inheritance	Genetic defect	Phenotypic defect
Severe congenital neutropenia	Autosomal dominant, autosomal recessive or sporadic	*ELANE* mutation *HAX1* mutation *GFI1* mutation	Severe neutropenia, developmental arrest of neutrophil precursors and maturational arrest at promyelocytic stage
Cyclic neutropenia	Autosomal dominant or sporadic	*ELANE* mutation	Fluctuations between normal granulocyte counts and severe neutropenia with 21-d periodicity. Increased apoptosis in myeloid precursors
Glucose-6-phosphatase deficiency	Autosomal recessive	*G6PC3* mutation	Loss of glucose-6-phosphatase activity and increased susceptibility to apoptosis
X-linked neutropenia	X-linked	Activating *WAS* mutation	Neutropenia and monocytopenia
Neutrophil specific granule deficiency	Autosomal recessive	*CEBPE* mutation	Absent secondary and tertiary granule proteins. Primary granules lack defensins
p14 deficiency	Autosomal recessive	*ROBLD3* (*p14*, *MAPBPIP*) mutation	Defective lysosome function, neutropenia, hypogammaglobulinaemia, short statue and hypopigmentation
Hermansky-Pudlak syndrome type 2	Autosomal recessive	*AP3B1* mutation	Neutropenia, hypopigmentation and defective platelet function
Griscelli syndrome type 2	Autosomal recessive	*RAB27A* mutation	Variable hypopigmentation, neutropenia and frequent pyogenic infections
Chediak-Higashi syndrome	Autosomal recessive	*LYST* (*CHS1*) mutation	Hypopigmentation, recurrent infections, defective bacterial killing and enlarged and irregular azurophillic granules

AP3B1, adaptor-related protein complex 3, beta 1 subunit; CEBPE, CCAAT/enhancer binding protein (C/EBP), epsilon; *LYST*, Lysosomal Trafficking Regulator (*CHS1*, Chediak-Higagshi Syndrome 1); *ELANE*, neutrophil elastase; *G6PC3*, glucose-6-phosphate catalytic subunit 3; *GFI1*, growth factor independent-1 transcription repressor; *HAX1*, HCLS1 associated protein X-1; *RAB27A*, RAB27A, member RAS oncogene family; *ROBLD3*, roadblock domain containing 3 (MAPBPIP, mitogen-activated protein-binding protein-interacting protein).

sis, enhanced transcription of normal ER-resident chaperone proteins involved in normal protein folding and degradation of misfolded proteins. Upon severe ER stress, the UPR triggers apoptosis (Ron & Walter, 2007). It has been suggested that elastase mutants induce the unfolded protein response. One study has shown that expression of several elastase mutants in a myeloid cell line resulted in disrupted intracellular trafficking and accumulation of cytoplasmic elastase. Furthermore, apoptosis was increased, which was associated with upregulated expression of the ER chaperone binding immunoglobulin protein (BiP) (Kollner *et al*, 2006). The second study confirmed increased BiP expression in the same myeloid cell line using additional elastase mutants, but importantly also observed increased BiP expression in granulocytic precursors isolated from patients with SCN (Grenda *et al*, 2007).

HAX1

While *ELANE* mutations account for around 50% of SCN cases, they appear not to be the cause of SCN in the original pedigree described by Kostmann (1956) as only one patient of this family was found to carry a mutation in the *ELANE* gene (Carlsson *et al*, 2006; Melin *et al*, 2007). Instead, three of the original kindred were found to have homozygous *HAX1* mutations (Klein *et al*, 2007). A small number of SCN patients have now been found to carry *HAX1* mutations with the W44X mutation being the most common (72% of cases) (Klein *et al*, 2007; Germeshausen *et al*, 2008; Smith *et al*, 2009). HAX1 deficient neutrophils showed enhanced spontaneous as well as induced apoptosis and while normal neutrophils maintain the inner mitochondrial membrane potential, HAX1 deficient neutrophils failed to do so, suggesting HAX1 is involved in stabilizing the mitochondrial membrane potential (Klein *et al*, 2007). Studies in HAX1-deficient mice have shown that HAX1 is required for processing of the mitochondrial protease high-temperature-regulated A2 (HtrA2), which is then activated and released into the mitochondrial intermembrane space and prevents accumulation of other pro-apoptotic factors (Chao *et al*, 2008). Loss of HtrA2 was shown to lead to an accumulation of unfolded proteins in mitochondria and enhanced mitochondrial stress in HtrA2-deficient mice (Moisoi *et al*, 2009). Defects of HAX1 or HtrA2 may result in failure to maintain normal mitochondria function and consequently induce apoptosis, but further investigations are required to determine if such mechanism is indeed involved in enhanced apoptosis of neutrophils in SCN caused by HAX1 deficiency.

Glucose-6-phosphatase (G6P) deficiency

Recently, mutations in the *G6PC3* gene, encoding glucose-6-phosphatase, catalytic subunit 3, were found to give rise to SCN. To date, a total of 15 SCN patients have been identified

to have mutations in *G6PC3* resulting in impaired enzymatic activity and an increased susceptibility to apoptosis (Arostegui *et al*, 2009; Boztug *et al*, 2009; Xia *et al*, 2009). Additional features include variable cardiac, urogenital and vascular anomalies. Analysis of neutrophil progenitor cells showed enlarged rough ER and increased BiP expression (Boztug *et al*, 2009), suggesting involvement of UPR as has been hypothesized for *ELANE* mutations causing SCN and cyclic neutropenia. Moreover, G6P-deficient mice display a SCN phenotype including neutropenia, increased susceptibility to bacterial infection, increased apoptosis and defects in neutrophil respiratory burst and chemotaxis (Cheung *et al*, 2007). Peritoneal and bone marrow neutrophils were found to exhibit enhanced expression of ER chaperones, suggesting *G6PC3*$^{-/-}$ neutrophils undergo ER stress and UPR leading to increased neutrophil apoptosis (Cheung *et al*, 2007).

X-linked neutropenia

Although loss of function mutations in *WAS*, the gene encoding the Wiskott–Aldrich syndrome (WAS) protein (WASp), normally result in classical WAS or X-linked thrombocytopenia, to date four different constitutively activating mutations have been described to cause a distinct clinical disease, X-linked neutropenia (XLN). XLN does not resemble WAS but is instead characterized by moderate-severe neutropenia, recurrent infections and in some cases myelodysplasia (Devriendt *et al*, 2001; Ancliff *et al*, 2006; Beel *et al*, 2009; Xia *et al*, 2009; Thrasher & Burns, 2010). It appears that mutations in the conserved GTPase-binding domain of WASp disrupt the autoinhibitory molecular confirmation of the cytosolic protein, resulting in mutant protein with enhanced Arp2/3-mediated actin polymerization activity (Devriendt *et al*, 2001; Ancliff *et al*, 2006; Moulding *et al*, 2007; Beel *et al*, 2009). The mechanism by which hyperactive WASp causes neutropenia remains to be fully elucidated, but recent data suggest that enhanced and delocalized actin polymerization disrupts normal mitosis and leads to genomic instability and increased apoptosis of neutrophil precursors (Moulding *et al*, 2007). Despite clinical heterogeneity, infection susceptibility is generally relatively mild given the degree of neutropenia and patients can usually be managed with antibiotic prophylaxis. Concern regarding myelodysplasia and an increased risk of malignant transformation associated with G-CSFR mutations and monosomy 7, has led to a recommendation that G-CSF is used with caution and ideally reserved for management of acute infections (Beel & Vandenberghe, 2009; Beel *et al*, 2009). A fifth mutation in *WAS* (P460S) has recently been described to be linked with neutropenia, but it is not known whether this mutation affects the ability of WASp to induce Arp2/3-mediated actin polymerization (Xia *et al*, 2009).

Myeloid transcription factors

During haematopoiesis transcription factors determine the regulation of gene expression required for cellular proliferation, differentiation and survival. Myeloid transcription factors, such as the alpha and epsilon members of the CCAAT/enhancer binding protein (C/EBP) family and PU.1 (encoded by the *CEBP* and *SPI1* genes respectively), are key regulators of granulocytic-macrophage progenitor cells and were, prior to the above discoveries, a key area of investigation. Germline mutations the *CEBPA* gene do not seem to give rise to SCN, but rather are associated with leukaemia (Smith *et al*, 2004). In contrast, mice lacking C/EBPα are unable to respond to G-CSF and as a result have a selective loss of granulocytes (Zhang *et al*, 1997). It has been proposed that the transcription factor lymphoid enhancer factor-1 (LEF-1) plays a key role in regulating neutrophil granulopoiesis by regulating C/EBPα (Skokowa & Welte, 2009). LEF-1 expression was found to be reduced in SCN patients carrying either *ELANE* or *HAX1* mutations, resulting in reduced expression of C/EBPα and neutrophil elastase (Skokowa *et al*, 2006, 2009). Reconstitution of LEF-1 in cells from SCN patients corrected their defective myelopoiesis and restored differentiation of mature neutrophils, while inhibition of LEF-1 in progenitor cells from healthy individuals inhibited their proliferation and induced apoptosis (Skokowa *et al*, 2006). However, no mutations in *LEF1* have yet been found in SCN patients.

C/EBPε consists of four isoforms and is expressed exclusively in cells of myeloid and T-cell lineages (Antonson *et al*, 1996). Targeted disruption of the *Cebpe* gene in mice results in failed terminal differentiation of neutrophils and eosinophils (Yamanaka *et al*, 1997). C/EBPε$^{-/-}$ neutrophils are defective in phagocytosis, bacterial killing and migration, and show impaired cytokine production after an inflammatory challenge (Yamanaka *et al*, 1997; Lekstrom-Himes & Xanthopoulos, 1999; Hock *et al*, 2003). In addition, there is accelerated apoptosis in maturing granulocytic cells (Verbeek *et al*, 2001). The phenotype of C/EBPε$^{-/-}$ mice is strikingly similar to a rare congenital neutrophil disorder neutrophil-specific granule deficiency (SGD). Five cases with this disease have been reported worldwide who suffer from frequent and severe bacterial infections and their neutrophils are characterized by bilobed nuclei and insufficient neutrophil function including lack of secondary granule proteins and defensins, impaired chemotaxis and impaired bactericidal activity (Gallin, 1985; Gombart & Koeffler, 2002). In two of these patients mutations in *CEBPE* have been found, resulting in loss of two or all four of the C/EBPε isoforms (Lekstrom-Himes *et al*, 1999; Gombart *et al*, 2001). A third patient was found to exhibit elevated expression of C/EBPε, caused by a heterozygous *CEBPE* mutation (Khanna-Gupta *et al*, 2007). While this mutation did not seem to affect C/EBPε function, expression of both C/EBPε and PU.1 was increased. Both transcription factors are targets for repression by the transcriptional repressor transcription factor growth factor independence-1 (Gfi-1) and its expression was found to be reduced in this SGD patient (Khanna-Gupta *et al*, 2007).

Gfi-1 is a zinc-fingered transcription factor, which regulates multiple target genes for neutrophil differentiation, including

CEBPE and *SPI1* (*PU.1*). Gene targeted *GFI1*$^{-/-}$ mice lack mature circulating neutrophils and are highly susceptible to bacterial infection (Hock *et al*, 2003). Two individuals were reported with mutations in the human *GFI1* gene and neutropenia. Such mutations result in loss of repression of *ELANE* and *CEBPE* expression, which may induce the UPR or directly impair the proliferation and survival of immature granulocytes (Person *et al*, 2003; Zhuang *et al*, 2006). Three other mutations in *GFI1* were detected in a large screen of SCN patients, although it is not known whether these are disease-causing and two of them were found in addition to mutations in *ELANE* (Xia *et al*, 2009). Targeted gene disruption of the transcription factor PU.1 in mice also resulted in neutropenia (amongst other haematopoietic abnormalities) and abnormal neutrophil development with production of only a few cells with neutrophil characteristics, which failed to differentiate into mature, functional neutrophils (McKercher *et al*, 1996; Anderson *et al*, 1998).

Other forms of neutropenia

For many SCN patients no underlying molecular cause has been identified and there are several disorders that exhibit neutropenia as part of a broader spectrum of symptoms (Table I). A mutation in the gene encoding endosomal adaptor protein 14 (p14), encoded by the *ROBLD3* gene, was found to cause neutropenia, while neutrophil maturation in the bone marrow was intact. To date, four patients from one family have been described with this mutation, who suffered from recurrent *Streptococcus pneumoniae* infection, hypogammaglobulinaemia, short stature and hypopigmented skin (Bohn *et al*, 2007). Patients showed substantially reduced protein expression, which resulted in defective lysosome function and delayed bacterial killing by neutrophils (Bohn *et al*, 2007). Other diseases that include neutropenia in their clinical presentation include Hermansky-Pudlak syndrome type 2, which is caused by mutations in *AP3B1* (Jung *et al*, 2006), Griscelli syndrome type 2, caused by mutations in the *RAB27A* gene, which encodes the GTPase Rab27 (Meeths *et al*, 2009) and the Chediak-Higashi syndrome, caused by mutations in the *LYST* (*CHS1*) gene (Introne *et al*, 1999). Patients with X-linked hyper IgM syndrome, which is characterized by CD40L deficiency, also frequently develop neutropenia and the mechanism may be related to reduced cytokine production from bone marrow stromal cells secondary to lack of CD40L stimulation (Solanilla *et al*, 2000).

Defects of migration and cytoskeletal function

Leucocyte adhesion disorders

Leucocyte adhesion disorders arise from defects in cell adhesion to extracellular matrix and vascular endothelium (Table II). These functions are normally mediated by leucocyte adhesion molecules including integrins and selectins (Fig 2). Integrins are classified in families where each member shares a common β chain that is non-covalently linked to a unique α chain. The β_2 integrins share CD18 as the β chain and are confined primarily to leucocytes. Depending on which α chain is utilized, they are known as lymphocyte-function-associated antigen (LFA-1 or CD11a/CD18), complement receptor-3 (CR3, Mac-1 or CD11b/CD18), p150,95 (or CD11c/CD18) and $\alpha v\beta_2$ (or CD11d/CD18). All β_2 integrins are expressed constitutively at the cell surface but are quantitatively and functionally upregulated by activation of the cell, to achieve optimal ligand binding. The ligands for β_2 integrins are members of the immunoglobulin gene superfamily, intercellular adhesion molecule-1 (ICAM-1) and ICAM-2, which are expressed on endothelial cells and upregulated during inflammation. Three distinct human disorders of leucocyte adhesion have been recognized: leucocyte adhesion deficiency type 1 (LAD-I) of which hundreds of cases are described, and the rarer LAD type 2 (LAD-II) and LAD type 3 (LAD-III).

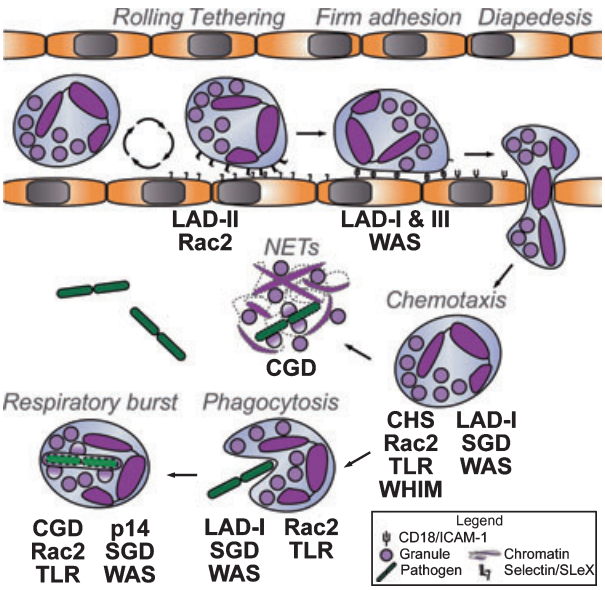

Fig 2. Schematic overview of normal neutrophil function and its defects. In response to inflammatory signals, e.g. caused by bacteria, neutrophils leave the blood stream and enter tissue via the multistep paradigm of rolling, tethering, adhesion and diapedesis. Inside the tissue neutrophils chemotax towards the source of inflammation and phagocytose and kill the bacteria using respiratory burst machinery and formation of neutrophil extracellular traps (NETs). One or more of these cellular processes can be disrupted by an inherited neutrophil defect. The genetic diseases associated with impairment of each functional stage are indicated in bold font. Abbreviations as in the main text.

LAD-I

LAD-I is characterized in its severe form by delayed separation of the umbilical cord with omphalitis, recurrent life-threatening

Table II. Disorders of neutrophil function.

Disease	Inheritance	Genetic defect	Phenotypic defect
Defects of migration and cytoskeletal function			
LAD-I	Autosomal recessive	*ITGB2* mutation	Neutrophilia, inability to make pus and lack or reduction of CD18 expression
LAD-II	Autosomal recessive	*SLC35C1* mutation	Granulocytes unable to bind to selectins on endothelium. Congenital disorder of fucosylation
LAD-III	Autosomal recessive	*RASGRP2* mutation *FERMT3* mutation	Neutrophilia, severe bleeding tendency and defective integrin activation
WHIM	Autosomal dominant or autosomal recessive	*CXCR4* mutation	Warts, hypogammaglobulinaemia, infections and myelokathexis
WAS	X-linked	*WAS* mutation	Dysfunctional actin polymerization and defects of adhesion, migration and phagocytosis
Rac2 deficiency	Autosomal dominant	*RAC2* mutation	Abnormal chemotaxis, defective respiratory burst, impaired degranulation and neutrophillia
Defects of the respiratory burst			
CGD	X-linked autosomal recessive	*CYBB* mutation *NCF1*, *NCF2*, *NCF4* and *CYBA* mutations	Neutrophils unable to produce superoxide
G6PD deficiency	X-linked	*G6PD* mutation	Neutrophils unable to produce superoxide
MPO deficiency	Multigenic?	*MPO* and other genetic mutations?	Impaired respiratory burst from defective hypochlorous acid production
Defects of TLR signalling			
IRAK-4 deficiency	Autosomal recessive	*IRAK4* mutation	Increased susceptibility to invasive bacterial disease, defective L-selectin shedding, migration and respiratory burst
MyD88 deficiency	Autosomal recessive	*MYD88* mutation	Increased susceptibility to invasive bacterial disease
NEMO deficiency	X-linked	*IKBKG* mutation	Increased susceptibility to invasive bacterial disease and impaired respiratory burst

CGD, chronic granulomatous disease; *CXCR4*, CXC-containing chemokine receptor-4; *CYBA*, cytochrome b-245 alpha polypeptide; *CYBB*, cytochrome b-245 beta polypeptide; *FERMT3*, fermitin family homolog 3; G6PD/*G6PD*, glucose-6-phosphate dehydrogenase; IRAK-4/*IRAK4*, interleukin-1 receptor-associated kinase 4; *IKBKG*, inhibitor of nuclear factor kappa-B kinase subunit gamma; *ITGB2*, integrin beta-2; LAD, leucocyte adhesion deficiency; MPO/*MPO*, myeloperoxidase; MyD88/*MYD88*, myeloid differentiation primary response gene 88; *NCF*, neutrophil cytosolic factor; NEMO, nuclear factor kB modulator; *RASGRP2*, RAS guanyl releasing protein-2; *SLC35C1*, solute carrier family 35 member C1; WAS/*WAS*, Wiskott–Aldrich syndrome; WHIM, warts, hypogammaglobulinaemia, infections and myelokathexis.

bacterial (usually with *Staphylococcus aureus* and Gram-negative enteric organisms) and fungal infections, gingivitis, and delayed wound healing. Patients are neutrophilic in the absence of infection with marked granulocytosis during acute infection. The absence of pus formation at sites of infection is one of the hallmarks of LAD-I, indicating a defect of neutrophil recruitment from the vasculature to sites of infection (Anderson & Springer, 1987; Etzioni *et al*, 1999). LAD-I is caused by recessively inherited mutations in *ITGB2*, encoding CD18, and is located on chromosome 21. All leucocytes are consequently deficient in CD18 cell-surface expression resulting in defects of neutrophil adhesion, transendothelial migration and chemotaxis (Anderson & Springer, 1987; Hogg & Bates, 2000; Tsai *et al*, 2008). The degree of CD18 deficiency correlates well with the severity of the clinical condition. Patients suffering from the severe form of the disease (<2% normal cell surface expression of CD18) are likely to die in childhood as a result of infection unless treated by haematopoietic stem cell transplantation, whereas those with moderate disease (2–10% expression) can survive into adulthood. Periodontitis, skin ulcerations and poor wound healing are common problems for those surviving past infancy. Although neutrophil defects appear to be the major clinical problem in LAD, the function of other immune cells is likely to be impaired given the universal expression of CD18 in leucocyte populations. Spontaneous reversion mutations, resulting in corrected copy of the gene have been described in a few patients (Uzel *et al*, 2008) but correction has so far been restricted to cytotoxic T-cells with no beneficial effect on host protection. Bone marrow transplantation is currently the only curative therapy available. In contrast with haploidentical procedures, which have a poor survival rate of about 50%, longterm survival is above 80% if a suitable human leucocyte antigen (HLA)-matched donor can be used (Qasim *et al*, 2009). Gene therapy has succesfully corrected a natural model of LAD-I in dogs (Bauer *et al*, 2008), and may in the future provide an alternative to stem cell transplantation for patients for whom no suitable donor can be found.

LAD-II

LAD-II has been described in only a few patients and results from an autosomal recessively inherited general defect in fucose metabolism leading to an absence of sialyl-Lewis X (SLeX, also called CD15s) and other fucosylated ligands for selectins (Etzioni *et al*, 1992; Marquardt *et al*, 1999a; Hidalgo *et al*, 2003). SLeX-containing glycoproteins on the endothelium normally bind the L-selectin cell-adhesion molecule on neutrophils, slowing them from the blood stream to establish rolling along endothelium. In LAD-II, this interaction is impaired abrogating firm integrin-mediated attachment and subsequent diapedesis (see Fig 2) and resulting in an immunodeficiency of LAD-II that is similar to that of LAD-I, but less severe. In addition to recurrent bacterial infections, other features include moderate-severe mental retardation, dysmorphism short stature and a Bombay blood phenotype. With advancing age, bacterial infections reduce in frequency and severity but periodontitis and mental retardation with epilepsy become more prominent. Missense mutations have been identified in *SLC35C1*, encoding a highly conserved GDP-fucose transporter, which explains the observed defect of GDP-fucose import into the Golgi apparatus where fucosylation takes place (Lubke *et al*, 1999, 2001; Luhn *et al*, 2001). *In vitro* fucosylation defects of cultured fibroblasts from LAD-II patients can be corrected by the addition of fucose (Marquardt *et al*, 1999b), suggesting that the defective transporter may have partial activity, or that other pathways for fucosylation exist. The clinical usefeulness of this is unclear however as oral fucose treatment restored expression of fucosylated selectin ligands and improved immunodeficiency in only one out of three LAD-II patients reported (Marquardt *et al*, 1999b; Etzioni & Tonetti, 2000; Luhn *et al*, 2001).

LAD-III

LAD-III was initially described as a variant of LAD-I, and presents a similar clinical phenotype including severe recurrent bacterial and fungal infections, leucocytosis, lack of pus formation and poor wound healing. In addition, however, affected individuals demonstrate a severe bleeding tendency, which is similar to Glanzmann thrombasthenia. Bleeding can be the presenting symptom and results in an ongoing requirement for red cell and platelet transfusions (Kuijpers *et al*, 1997). Hepatosplenomegally, possibly resulting from extra-medullary haematopoiesis, and an increase in bone density resembling osteopetrosis have also been reported (Kuijpers *et al*, 2007; Kilic & Etzioni, 2009). Patients have normal β_1, β_2 and β_3-integrin expression, but defective integrin activation through 'inside-out' signalling pathways, which results in failure to establish high affinity ligand binding (McDowall *et al*, 2003; Kinashi *et al*, 2004). Although mutations in *RASGRP2*, which encodes for calcium- and diacylglycerol-regulated guanine exchange factor (CalDAG-GEFI), were initially identified and implicated in LAD-III (Pasvolsky *et al*, 2007), further analysis has shown that these mutations are probably not disease-causing (Kuijpers *et al*, 2009; Svensson *et al*, 2009). Instead, separate recessively inherited mutations in *FERMT3*, which encodes the haematopoietic-restricted Kindlin-3 protein, were identified in LAD-III patients (Mory *et al*, 2008; Kuijpers *et al*, 2009; Svensson *et al*, 2009). All mutations described abrogate expression of Kindlin-3 protein, which normally functions to activate leucocyte integrins for optimal function. Restoring Kindlin-3 protein expression in affected cells of patients with LAD-III restored integrin-mediated adhesion and migration defects, directly implicating Kindlin-3 mutations in this disease (Svensson *et al*, 2009). Bone marrow transplantation (BMT) can be curative in LAD-III although it remains to be clarified whether engraftment failure and post BMT mortality are specifically problematic (Kuijpers *et al*, 2009; Elhasid *et al*, 2010; Jurk *et al*, 2010; Sabnis *et al*, 2010).

WHIM

WHIM syndrome is classically characterized by the clinical features of warts, hypogammaglobulinaemia, recurrent respiratory tract infections and myelokathexis, although affected individuals may not present all features (Tassone *et al*, 2009). Almost all patients carry dominant mutations in *CRCX4*, which encodes the chemokine receptor CXCR4, typically resulting in expression of a truncated form of CXCR4 that lacks its intracellular tail (Hernandez *et al*, 2003; Balabanian *et al*, 2005; Tassone *et al*, 2009). This causes alterations of CXCR4 signalling, and possibly receptor internalization, resulting in augmented responses to CXCL12, the functional ligand of CXCR4 (Gulino *et al*, 2004; Balabanian *et al*, 2005; Lagane *et al*, 2008). This is thought to attenuate neutrophil migration causing retention of mature neutrophils in the bone marrow (myelokathexis) and enhanced bone marrow homing of senescent neutrophils (Martin *et al*, 2003). In general, WHIM syndrome is not considered to be a severe immunodeficiency and can be managed with G-CSF, prophylactic antibiotics and immunoglobulin replacement. However, respiratory infections can lead to chronic lung disease and there is a significant malignancy susceptibility, particularly to human papillomavirus (HPV) related carcinomas (Kawai & Malech, 2009).

Wiskott–Aldrich syndrome

Dynamic reorganization of the actin cytoskeleton is a key process during many neutrophil functions including diapedesis, migration and phagocytosis. In haematopoietic cells, WASp is an important regulator of cytoskeletal function, transducing signals from cell surface receptors to the actin cytoskeleton. Loss of function mutations in the *WAS* gene give rise to the rare inherited X-linked Wiskott–Aldrich syndrome (WAS), which is characterized in its classic form by recurrent infections, eczema and microthrombocytopenia (Bouma *et al*,

2009a; Thrasher & Burns, 2010). The immunodeficiency seen in severe WAS is broad, affecting cells of both the innate and adaptive immune systems and this is reflected in the range of infections seen which include bacterial, viral, fungal and occasionally opportunistic pathogens. Although WAS is not considered to be a neutrophil disorder and indeed clinical features of neutrophil dysfunction are not prominent, specific defects have been reported *in vitro*. WASp-deficient neutrophils are impaired in their ability to cluster β2 integrins, which results in defective adhesion and transendothelial migration under conditions of physiological shear flow (Zicha *et al*, 1998; Zhang *et al*, 2006). In addition, phagocytosis and respiratory burst are impaired in neutrophils from patients and the murine WASp knockout model (Zhang *et al*, 1999, 2006).

Rac2

The GTPase Rac2 is another important regulator of leucocyte cytoskeletal rearrangement but is also critical for activity of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, through interaction with $p67^{phox}$ (see below) (Segal & Abo, 1993). Human Rac2 deficiency has been described in one patient presenting in infancy with a picture similar to LAD: severe bacterial infections, neutrophilia, absent pus formation and wound healing. A point mutation was found in the *RAC2* gene (Ambruso *et al*, 2000; Williams *et al*, 2000) which resulted in loss of GTP binding and dominant inhibition of Rac2 protein function (Gu *et al*, 2001). In keeping with dual roles in cytoskeletal regulation and respiratory burst, the patient's neutrophils exhibited decreased chemotaxis, polarization, azurophilic granule secretion and superoxide anion production. This patient underwent successful bone marrow transplantation (Williams *et al*, 2000).

Defects of the respiratory burst

Chronic granulomatous disease

Although still rare, chronic granulomatous disease (CGD) is the most common of the neutrophil function disorders with an estimated prevalence of approximately 4–8 per million (Winkelstein *et al*, 2000; Jones *et al*, 2008). CGD is caused by defects in any component of NADPH oxidase, the enzyme responsible for the oxidative or 'respiratory' burst in neutrophils (Goldblatt & Thrasher, 2000; Segal, 2005). The NADPH oxidase is a membrane-bound complex that is assembled in phagosomes or at the plasma membrane upon neutrophil activation by receptor binding or soluble factors. The main redox component is membrane-bound flavocytochrome b_{558}, a heterodimer of two proteins: the α subunit, $p22^{phox}$ and the β subunit, $gp91^{phox}$. Both $p22^{phox}$ and $gp91^{phox}$ are missing in cells derived from most CGD patients with a molecular lesion of either subunit, indicating that mutual interaction is required for the assembly of the mature flavocytochrome b_{558} complex (Parkos *et al*, 1989). The other subunits of the NADPH oxidase complex are four factors recruited from the cytosol, $p47^{phox}$, $p67^{phox}$, $p40^{phox}$ and p21rac2 (Segal, 2005), inducing conformational changes in flavocytochrome b_{558}, that permit binding of the substrate NADPH and energetically favour electron transport (Bedard & Krause, 2007). Once assembled, NADPH-oxidase catalyses the transfer of electrons across the phagosome membrane from NADPH to molecular oxygen, resulting in the formation of free radical superoxide (O_2^-) and subsequently hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and hydroxyl radical (·OH). Although reactive oxygen species have been supposed to have significant intrinsic microbicidal activity it appears more likely that the process of electron transport ultimately regulates pH and ionic composition within the phagosome, which is crucial for activation of proteolytic enzymes as they are discharged into the phagocytic vacuole (Segal, 2005).

Patients affected by CGD lack specific components of the NADPH oxidase complex as a result of mutations in corresponding genes. X-linked CGD affecting $gp91^{phox}$ (*CYBB* gene) is the most common and accounts for about 70% of cases. Mutations affecting the other components demonstrate autosomal recessive inheritance: approximately 25% of cases result from mutations in $p47^{phox}$ (*NCF1* gene), while loss of $p67^{phox}$ (*NCF2* gene) or $p22^{phox}$ (*CYBA* gene) account for only a small number of cases (each around 5%) (van den Berg *et al*, 2009). In all these forms of CGD, failure to produce a respiratory burst results in susceptibility to severe and recurrent infections predominantly by catalase-positive bacteria and fungi, particularly *Aspergillus* species, *S. aureus* and *Salmonella* species. In addition to defective respiratory burst, CGD neutrophils also fail to form NETs (Fuchs *et al*, 2007), which were recently found to provide an important additional antimicrobial mechanism for neutrophils to bind and kill bacteria. Lymphadenitis, osteomyelitis, absecesses and septicaemia are common but pulmonary infections are most frequent and result in chronic lung disease in a high proportion of patients (Winkelstein *et al*, 2000; Jones *et al*, 2008; van den Berg *et al*, 2009). Granulomatous inflammation is the other major feature of CGD, particularly affecting hollow organs, such as the gastrointestinal and genitourinary tracts. This can result in obstruction or more commonly Crohn's-like inflammatory bowel disease, which may occur early in life and be the sole presenting feature (Freudenberg *et al*, 2010). There is an increased incidence of autoinflammatory manifestations including chorio-retinitis and discoid lupus, which can also be seen in carriers of X-linked CGD (De Ravin *et al*, 2008; van den Berg *et al*, 2009). In general, autosomal recessive forms of CGD have a milder disease course than X-linked CGD, although this is more difficult to establish for the rare $p67^{phox}$ or $p22^{phox}$ forms. Within each subtype, there is significant clinical heterogeneity which, to date, has not been correlated with the specific type of mutation. Recently, the first patient bearing a mutation in $p40^{phox}$ (*NCF4* gene) was described presenting with granulomatous colitis (Matute *et al*, 2009). Loss of $p40^{phox}$ function differs from other forms of CGD as

superoxide production in response to phorbol ester is normal and the substantial defect in superoxide release is seen only during phagocytosis (Matute *et al*, 2009).

The diagnosis of CGD and the detection of carrier status are based on measurement of superoxide release in response to neutrophil activation by one of a variety of tests including nitroblue tetrazolium reduction, dihydrorhodamine (DHR) oxidation or chemiluminscence. Particular consideration needs to be given to myeloperoxidase (MPO) deficiency which also results in an abnormal DHR response but normal NBT test and to $p40^{phox}$ CGD in which superoxide release is only abnormal when phagocytosis is used as an activation stimulus.

Treatment for CGD is dependent on prophylaxis against both bacterial and fungal infection with antimicrobials. In general in Europe, interferon gamma does not form part of the prophylactic arsenal but is reserved for treatment of acute infection. Despite prophylaxis, longterm survival is significantly reduced to approximately 50–60% in the fourth decade of life for X-linked disease and around 80% for autosomal recessive forms (Jones *et al*, 2008; van den Berg *et al*, 2009). Haematopoietic stem cell transplantation is potentially curative and may be considered for patients who have had life threatening infections or severe inflammatory complications, particularly if an HLA-matched sibling donor is available. Clinical trials of gene therapy of X-linked CGD have been in progress for some years and although long-term cure has not yet been achieved, significant clinical benefit in terms of clearing life-threatening infection has been reported (Ott *et al*, 2006; Stein *et al*, 2010). Challenges for the future include establishing long term gene correction and designing new vectors to avoid complications such as retrovirus-induced insertional mutagenesis which has induced myelodysplasia in one trial (Ott *et al*, 2006; Stein *et al*, 2010).

Other defects of oxidative metabolism

Glucose-6-phosphate dehydrogenase (G6PD) deficiency causes defects in oxidative metabolism as G6PD is required for the reduction of NADP+ to NADPH. Better known for causing haemolysis, G6PD deficiency can also result in an increased susceptibility to infection. However, as G6PD activity needs to be <5% to cause such symptoms, only a tiny proportion of individuals have this complication (Baehner *et al*, 1971). MPO is a component of neutrophil granules and normally functions to generate antimicrobial hypochlorous acid. This is lacking in MPO deficiency and although the majority of affected individuals are asymptomatic, a small proportion suffer from recurrent mucosal, skin or invasive Candida infections (Lanza, 1998).

Defects of TLR signalling

Toll-like receptors (TLRs) have been recognized as key leucocyte receptors for recognition of pathogen-associated molecules. Their ligation, with the exception of TLR3, results in signalling through the MyD88 adapter molecule and recruitment of the IL-1R-associated kinase (IRAK) complex to regulate activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPKs) signalling leading to release of pro-inflammatory cytokines and initiation of antimicrobial effector functions. Defects of TLR signalling, as seen in human deficiency of the IRAK-4 subunit, cause susceptibility to invasive bacterial disease, although curiously this improves with age (Ku *et al*, 2005, 2007). Several leucocyte subtypes demonstrate impaired inflammatory responses and neutrophils of patients with IRAK-4 deficiency show defective shedding of the rolling/tethering receptor L-selectin, defective migration, variable defects of phagocytosis and impaired priming of formyl-methionyl-leucyl-phenylalanine (fMLP)-induced respiratory burst in response to a variety of TLR ligands (Medvedev *et al*, 2003; Picard *et al*, 2003; von Bernuth *et al*, 2006; Ku *et al*, 2007; Bouma *et al*, 2009b; Singh *et al*, 2009; van Bruggen *et al*, 2010). Patients with MyD88 deficiency have been reported with an indistinguishable cellular phenotype from IRAK-4 deficiency and it is likely that neutrophil function will be similarly defective, but this was not investigated (von Bernuth *et al*, 2008). Additionally, patients with NF-κB essential modulator (NEMO) deficiency, who exhibit mutations in the regulatory complex that controls degradation of IκB and thus NF-κB activation, show impaired priming of the neutrophil respiratory burst, although not as severe as IRAK-4 deficient neutrophils (Singh *et al*, 2009).

Concluding remarks

Recent advances in defining the molecular basis of neutrophil defects have significantly enhanced our understanding of these disorders and will enable improved studies of disease outcome and the effect of current therapies. In contrast with congenital neutropenias, in which many different underlying mutations result in similar final effects on neutrophil differentiation and production, defects of neutrophil function are disparate genetically, biochemically and clinically. While G-CSF is the mainstay of treatment for genetic neutropenias, a variety of treatment modalities from prophylactic antimicrobials to stem cell transplantation are appropriate for functional disorders. In addition to the defined disorders discussed here, a significant proportion of individuals with infection susceptibility and demonstrable neutrophil dysfunction remain undefined. This group presents a significant challenge for the future, both to determine underlying molecular mechanisms and optimum treatment. Future challenges, which need to be addressed, are improved diagnostic testing for neutrophil function and novel therapeutic options, such as gene therapy, for patients for whom no suitable donor is available for stem cell transplantation.

References

Ambruso, D.R., Knall, C., Abell, A.N., Panepinto, J., Kurkchubasche, A., Thurman, G., Gonzalez-Aller, C., Hiester, A., deBoer, M., Harbeck, R.J., Oyer, R., Johnson, G.L. & Roos, D. (2000) Human

neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation. *Proceedings of the National Academy of Sciences of the United States of America*, **97,** 4654–4659.

Ancliff, P.J., Gale, R.E., Liesner, R., Hann, I.M. & Linch, D.C. (2001) Mutations in the ELA2 gene encoding neutrophil elastase are present in most patients with sporadic severe congenital neutropenia but only in some patients with the familial form of the disease. *Blood*, **98,** 2645–2650.

Ancliff, P.J., Gale, R.E., Liesner, R., Hann, I. & Linch, D.C. (2003) Long-term follow-up of granulocyte colony-stimulating factor receptor mutations in patients with severe congenital neutropenia: implications for leukaemogenesis and therapy. *British Journal of Haematology*, **120,** 685–690.

Ancliff, P.J., Blundell, M.P., Cory, G.O., Calle, Y., Worth, A., Kempski, H., Burns, S., Jones, G.E., Sinclair, J., Kinnon, C., Hann, I.M., Gale, R.E., Linch, D.C. & Thrasher, A.J. (2006) Two novel activating mutations in the Wiskott–Aldrich syndrome protein result in congenital neutropenia. *Blood*, **108,** 2182–2189.

Anderson, D.C. & Springer, T.A. (1987) Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annual Review of Medicine*, **38,** 175–194.

Anderson, K.L., Smith, K.A., Pio, F., Torbett, B.E. & Maki, R.A. (1998) Neutrophils deficient in PU.1 do not terminally differentiate or become functionally competent. *Blood*, **92,** 1576–1585.

Antonson, P., Stellan, B., Yamanaka, R. & Xanthopoulos, K.G. (1996) A novel human CCAAT/enhancer binding protein gene, C/EBPepsilon, is expressed in cells of lymphoid and myeloid lineages and is localized on chromosome 14q11.2 close to the T-cell receptor alpha/delta locus. *Genomics*, **35,** 30–38.

Arostegui, J.I., de Toledo, J.S., Pascal, M., Garcia, C., Yague, J. & Diaz de Heredia, C. (2009) A novel G6PC3 homozygous 1-bp deletion as a cause of severe congenital neutropenia. *Blood*, **114,** 1718–1719.

Baehner, R.L., Nathan, D.G. & Castle, W.B. (1971) Oxidant injury of caucasian glucose-6-phosphate dehydrogenase-deficient red blood cells by phagocytosing leukocytes during infection. *Journal of Clinical Investigation*, **50,** 2466–2473.

Balabanian, K., Lagane, B., Pablos, J.L., Laurent, L., Planchenault, T., Verola, O., Lebbe, C., Kerob, D., Dupuy, A., Hermine, O., Nicolas, J.F., Latger-Cannard, V., Bensoussan, D., Bordigoni, P., Baleux, F., Le Deist, F., Virelizier, J.L., Arenzana-Seisdedos, F. & Bachelerie, F. (2005) WHIM syndromes with different genetic anomalies are accounted for by impaired CXCR4 desensitization to CXCL12. *Blood*, **105,** 2449–2457.

Bauer, Jr, T.R., Allen, J.M., Hai, M., Tuschong, L.M., Khan, I.F., Olson, E.M., Adler, R.L., Burkholder, T.H., Gu, Y.C., Russell, D.W. & Hickstein, D.D. (2008) Successful treatment of canine leukocyte adhesion deficiency by foamy virus vectors. *Nature Medicine*, **14,** 93–97.

Bedard, K. & Krause, K.H. (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiological Reviews*, **87,** 245–313.

Beel, K. & Vandenberghe, P. (2009) G-CSF receptor (CSF3R) mutations in X-linked neutropenia evolving to acute myeloid leukemia or myelodysplasia. *Haematologica*, **94,** 1449–1452.

Beel, K., Cotter, M.M., Blatny, J., Bond, J., Lucas, G., Green, F., Vanduppen, V., Leung, D.W., Rooney, S., Smith, O.P., Rosen, M.K. & Vandenberghe, P. (2009) A large kindred with X-linked neutropenia with an I294T mutation of the Wiskott–Aldrich syndrome gene. *British Journal of Haematology*, **144,** 120–126.

Belaaouaj, A., McCarthy, R., Baumann, M., Gao, Z., Ley, T.J., Abraham, S.N. & Shapiro, S.D. (1998) Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. *Nature Medicine*, **4,** 615–618.

Bellanne-Chantelot, C., Clauin, S., Leblanc, T., Cassinat, B., Rodrigues-Lima, F., Beaufils, S., Vaury, C., Barkaoui, M., Fenneteau, O., Maier-Redelsperger, M., Chomienne, C. & Donadieu, J. (2004) Mutations in the ELA2 gene correlate with more severe expression of neutropenia: a study of 81 patients from the French Neutropenia Register. *Blood*, **103,** 4119–4125.

van den Berg, J.M., van Koppen, E., Ahlin, A., Belohradsky, B.H., Bernatowska, E., Corbeel, L., Espanol, T., Fischer, A., Kurenko-Deptuch, M., Mouy, R., Petropoulou, T., Roesler, J., Seger, R., Stasia, M.J., Valerius, N.H., Weening, R.S., Wolach, B., Roos, D. & Kuijpers, T.W. (2009) Chronic granulomatous disease: the European experience. *PLoS ONE*, **4,** e5234.

von Bernuth, H., Ku, C.L., Rodriguez-Gallego, C., Zhang, S., Garty, B.Z., Marodi, L., Chapel, H., Chrabieh, M., Miller, R.L., Picard, C., Puel, A. & Casanova, J.L. (2006) A fast procedure for the detection of defects in Toll-like receptor signaling. *Pediatrics*, **118,** 2498–2503.

von Bernuth, H., Picard, C., Jin, Z., Pankla, R., Xiao, H., Ku, C.L., Chrabieh, M., Mustapha, I.B., Ghandil, P., Camcioglu, Y., Vasconcelos, J., Sirvent, N., Guedes, M., Vitor, A.B., Herrero-Mata, M.J., Arostegui, J.I., Rodrigo, C., Alsina, L., Ruiz-Ortiz, E., Juan, M., Fortuny, C., Yague, J., Anton, J., Pascal, M., Chang, H.H., Janniere, L., Rose, Y., Garty, B.Z., Chapel, H., Issekutz, A., Marodi, L., Rodriguez-Gallego, C., Banchereau, J., Abel, L., Li, X., Chaussabel, D., Puel, A. & Casanova, J.L. (2008) Pyogenic bacterial infections in humans with MyD88 deficiency. *Science*, **321,** 691–696.

Bode, W., Meyer, Jr, E. & Powers, J.C. (1989) Human leukocyte and porcine pancreatic elastase: X-ray crystal structures, mechanism, substrate specificity, and mechanism-based inhibitors. *Biochemistry*, **28,** 1951–1963.

Bohn, G., Allroth, A., Brandes, G., Thiel, J., Glocker, E., Schaffer, A.A., Rathinam, C., Taub, N., Teis, D., Zeidler, C., Dewey, R.A., Geffers, R., Buer, J., Huber, L.A., Welte, K., Grimbacher, B. & Klein, C. (2007) A novel human primary immunodeficiency syndrome caused by deficiency of the endosomal adaptor protein p14. *Nature Medicine*, **13,** 38–45.

Bouma, G., Burns, S.O. & Thrasher, A.J. (2009a) Wiskott–Aldrich syndrome: immunodeficiency resulting from defective cell migration and impaired immunostimulatory activation. *Immunobiology*, **214,** 778–790.

Bouma, G., Doffinger, R., Patel, S.Y., Peskett, E., Sinclair, J.C., Barcenas-Morales, G., Cerron-Gutierrez, L., Kumararatne, D.S., Graham Davies, E., Thrasher, A.J. & Burns, S.O. (2009b) Impaired neutrophil migration and phagocytosis in IRAK-4 deficiency. *British Journal of Haematology*, **147,** 153–156.

Boztug, K., Appaswamy, G., Ashikov, A., Schaffer, A.A., Salzer, U., Diestelhorst, J., Germeshausen, M., Brandes, G., Lee-Gossler, J., Noyan, F., Gatzke, A.K., Minkov, M., Greil, J., Kratz, C., Petropoulou, T., Pellier, I., Bellanne-Chantelot, C., Rezaei, N., Monkemoller, K., Irani-Hakimeh, N., Bakker, H., Gerardy-Schahn, R., Zeidler, C., Grimbacher, B., Welte, K. & Klein, C. (2009) A syndrome with congenital neutropenia and mutations in G6PC3. *New England Journal of Medicine*, **360,** 32–43.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., Weinrauch, Y. & Zychlinsky, A. (2004) Neutrophil extracellular traps kill bacteria. *Science*, **303,** 1532–1535.

van Bruggen, R., Drewniak, A., Tool, A.T., Jansen, M., van Houdt, M., Geissler, J., van den Berg, T.K., Chapel, H. & Kuijpers, T.W. (2010) Toll-like receptor responses in IRAK-4-deficient neutrophils. *Journal of Innate Immunity*, **2,** 280–287.

Carlsson, G., Aprikyan, A.A., Ericson, K.G., Stein, S., Makaryan, V., Dale, D.C., Nordenskjold, M., Fadeel, B., Palmblad, J. & Hentera, J.I. (2006) Neutrophil elastase and granulocyte colony-stimulating factor receptor mutation analyses and leukemia evolution in severe congenital neutropenia patients belonging to the original Kostmann family in northern Sweden. *Haematologica*, **91,** 589–595.

Cassinat, B., Bellanne-Chantelot, C., Notz-Carrere, A., Menot, M.L., Vaury, C., Micheau, M., Bader-Meunier, B., Perel, Y., Leblanc, T., Donadieu, J. & Chomienne, C. (2004) Screening for G-CSF receptor mutations in patients with secondary myeloid or lymphoid transformation of severe congenital neutropenia. A report from the French neutropenia register. *Leukemia*, **18,** 1553–1555.

Chao, J.R., Parganas, E., Boyd, K., Hong, C.Y., Opferman, J.T. & Ihle, J.N. (2008) Hax1-mediated processing of HtrA2 by Parl allows survival of lymphocytes and neurons. *Nature*, **452,** 98–102.

Cheung, Y.Y., Kim, S.Y., Yiu, W.H., Pan, C.J., Jun, H.S., Ruef, R.A., Lee, E.J., Westphal, H., Mansfield, B.C. & Chou, J.Y. (2007) Impaired neutrophil activity and increased susceptibility to bacterial infection in mice lacking glucose-6-phosphatase-beta. *Journal of Clinical Investigation*, **117,** 784–793.

Dale, D.C. & Hammond, W.P.t. (1988) Cyclic neutropenia: a clinical review. *Blood Reviews*, **2,** 178–185.

Dale, D.C., Bonilla, M.A., Davis, M.W., Nakanishi, A.M., Hammond, W.P., Kurtzberg, J., Wang, W., Jakubowski, A., Winton, E. & Lalezari, P. (1993) A randomized controlled phase III trial of recombinant human granulocyte colony-stimulating factor (filgrastim) for treatment of severe chronic neutropenia. *Blood*, **81,** 2496–2502.

Dale, D.C., Person, R.E., Bolyard, A.A., Aprikyan, A.G., Bos, C., Bonilla, M.A., Boxer, L.A., Kannourakis, G., Zeidler, C., Welte, K., Benson, K.F. & Horwitz, M. (2000) Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. *Blood*, **96,** 2317–2322.

Dale, D.C., Bolyard, A.A., Schwinzer, B.G., Pracht, G., Bonilla, M.A., Boxer, L., Freedman, M.H., Donadieu, J., Kannourakis, G., Alter, B.P., Cham, B.P., Winkelstein, J., Kinsey, S.E., Zeidler, C. & Welte, K. (2006) The Severe Chronic Neutropenia International Registry: 10-year follow-up report. *Supportive Cancer Therapy*, **3,** 220–231.

De Ravin, S.S., Naumann, N., Cowen, E.W., Friend, J., Hilligoss, D., Marquesen, M., Balow, J.E., Barron, K.S., Turner, M.L., Gallin, J.I. & Malech, H.L. (2008) Chronic granulomatous disease as a risk factor for autoimmune disease. *Journal of Allergy and Clinical Immunology*, **122,** 1097–1103.

Devriendt, K., Kim, A.S., Mathijs, G., Frints, S.G., Schwartz, M., Van Den Oord, J.J., Verhoef, G.E., Boogaerts, M.A., Fryns, J.P., You, D., Rosen, M.K. & Vandenberghe, P. (2001) Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. *Nature Genetics*, **27,** 313–317.

Dong, F., Brynes, R.K., Tidow, N., Welte, K., Lowenberg, B. & Touw, I.P. (1995) Mutations in the gene for the granulocyte colony-stimulating-factor receptor in patients with acute myeloid leukemia preceded by severe congenital neutropenia. *New England Journal of Medicine*, **333,** 487–493.

Elhasid, R., Kilic, S.S., Ben-Arush, M., Etzioni, A. & Rowe, J.M. (2010) Prompt recovery of recipient hematopoiesis after two consecutive haploidentical peripheral blood SCTs in a child with leukocyte adhesion defect III syndrome. *Bone Marrow Transplantation*, **45,** 413–414.

Etzioni, A. & Tonetti, M. (2000) Fucose supplementation in leukocyte adhesion deficiency type II. *Blood*, **95,** 3641–3643.

Etzioni, A., Frydman, M., Pollack, S., Avidor, I., Phillips, M.L., Paulson, J.C. & Gershoni-Baruch, R. (1992) Brief report: recurrent severe infections caused by a novel leukocyte adhesion deficiency. *New England Journal of Medicine*, **327,** 1789–1792.

Etzioni, A., Doerschuk, C.M. & Harlan, J.M. (1999) Of man and mouse: leukocyte and endothelial adhesion molecule deficiencies. *Blood*, **94,** 3281–3288.

Freedman, M.H. & Alter, B.P. (2002) Risk of myelodysplastic syndrome and acute myeloid leukemia in congenital neutropenias. *Seminars in Hematology*, **39,** 128–133.

Freudenberg, F., Wintergerst, U., Roesen-Wolff, A., Albert, M.H., Prell, C., Strahm, B., Koletzko, S., Ehl, S., Roos, D., Tommasini, A., Ventura, A., Belohradsky, B.H., Seger, R., Roesler, J. & Gungor, T. (2010) Therapeutic strategy in p47-phox deficient chronic granulomatous disease presenting as inflammatory bowel disease. *Journal of Allergy and Clinical Immunology*, **125,** 943–946.

Fuchs, T.A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahn, V., Weinrauch, Y., Brinkmann, V. & Zychlinsky, A. (2007) Novel cell death program leads to neutrophil extracellular traps. *Journal of Cell Biology*, **176,** 231–241.

Gallin, J.I. (1985) Neutrophil specific granule deficiency. *Annual Review of Medicine*, **36,** 263–274.

Germeshausen, M., Grudzien, M., Zeidler, C., Abdollahpour, H., Yetgin, S., Rezaei, N., Ballmaier, M., Grimbacher, B., Welte, K. & Klein, C. (2008) Novel HAX1 mutations in patients with severe congenital neutropenia reveal isoform-dependent genotype–phenotype associations. *Blood*, **111,** 4954–4957.

Germeshausen, M., Zeidler, C., Stuhrmann, M., Lanciotti, M., Ballmaier, M. & Welte, K. (2010) Digenic mutations in severe congenital neutropenia. *Haematologica*, **95,** 1207–1210.

Goldblatt, D. & Thrasher, A.J. (2000) Chronic granulomatous disease. *Clinical and Experimental Immunology*, **122,** 1–9.

Gombart, A.F. & Koeffler, H.P. (2002) Neutrophil specific granule deficiency and mutations in the gene encoding transcription factor C/EBP(epsilon). *Current Opinion in Hematology*, **9,** 36–42.

Gombart, A.F., Shiohara, M., Kwok, S.H., Agematsu, K., Komiyama, A. & Koeffler, H.P. (2001) Neutrophil-specific granule deficiency: homozygous recessive inheritance of a frameshift mutation in the gene encoding transcription factor CCAAT/enhancer binding protein–epsilon. *Blood*, **97,** 2561–2567.

Grenda, D.S., Johnson, S.E., Mayer, J.R., McLemore, M.L., Benson, K.F., Horwitz, M. & Link, D.C. (2002) Mice expressing a neutrophil elastase mutation derived from patients with severe congenital neutropenia have normal granulopoiesis. *Blood*, **100,** 3221–3228.

Grenda, D.S., Murakami, M., Ghatak, J., Xia, J., Boxer, L.A., Dale, D., Dinauer, M.C. & Link, D.C. (2007) Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. *Blood*, **110,** 4179–4187.

Gu, Y., Jia, B., Yang, F.C., D'Souza, M., Harris, C.E., Derrow, C.W., Zheng, Y. & Williams, D.A. (2001) Biochemical and biological characterization of a human Rac2 GTPase mutant associated with phagocytic immunodeficiency. *Journal of Biological Chemistry*, **276,** 15929–15938.

Gulino, A.V., Moratto, D., Sozzani, S., Cavadini, P., Otero, K., Tassone, L., Imberti, L., Pirovano, S., Notarangelo, L.D., Soresina, R., Mazzolari, E., Nelson, D.L. & Badolato, R. (2004) Altered leukocyte response to CXCL12 in patients with warts hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome. *Blood*, **104,** 444–452.

Haurie, C., Dale, D.C. & Mackey, M.C. (1998) Cyclical neutropenia and other periodic hematological disorders: a review of mechanisms and mathematical models. *Blood*, **92,** 2629–2640.

Hernandez, P.A., Gorlin, R.J., Lukens, J.N., Taniuchi, S., Bohinjec, J., Francois, F., Klotman, M.E. & Diaz, G.A. (2003) Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nature Genetics*, **34,** 70–74.

Hidalgo, A., Ma, S., Peired, A.J., Weiss, L.A., Cunningham-Rundles, C. & Frenette, P.S. (2003) Insights into leukocyte adhesion deficiency type 2 from a novel mutation in the GDP-fucose transporter gene. *Blood*, **101,** 1705–1712.

Hock, H., Hamblen, M.J., Rooke, H.M., Traver, D., Bronson, R.T., Cameron, S. & Orkin, S.H. (2003) Intrinsic requirement for zinc finger transcription factor Gfi-1 in neutrophil differentiation. *Immunity*, **18,** 109–120.

Hogg, N. & Bates, P.A. (2000) Genetic analysis of integrin function in man: LAD-1 and other syndromes. *Matrix Biology*, **19,** 211–222.

Horwitz, M., Benson, K.F., Person, R.E., Aprikyan, A.G. & Dale, D.C. (1999) Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. *Nature Genetics*, **23,** 433–436.

Horwitz, M.S., Duan, Z., Korkmaz, B., Lee, H.H., Mealiffe, M.E. & Salipante, S.J. (2007) Neutrophil elastase in cyclic and severe congenital neutropenia. *Blood*, **109,** 1817–1824.

Introne, W., Boissy, R.E. & Gahl, W.A. (1999) Clinical, molecular, and cell biological aspects of Chediak-Higashi syndrome. *Molecular Genetics and Metabolism*, **68,** 283–303.

Jones, L.B., McGrogan, P., Flood, T.J., Gennery, A.R., Morton, L., Thrasher, A., Goldblatt, D., Parker, L. & Cant, A.J. (2008) Special article: chronic granulomatous disease in the United Kingdom and Ireland: a comprehensive national patient-based registry. *Clinical and Experimental Immunology*, **152,** 211–218.

Jung, J., Bohn, G., Allroth, A., Boztug, K., Brandes, G., Sandrock, I., Schaffer, A.A., Rathinam, C., Kollner, I., Beger, C., Schilke, R., Welte, K., Grimbacher, B. & Klein, C. (2006) Identification of a homozygous deletion in the AP3B1 gene causing Hermansky-Pudlak syndrome, type 2. *Blood*, **108,** 362–369.

Jurk, K., Schulz, A.S., Kehrel, B.E., Rapple, D., Schulze, H., Mobest, D., Friedrich, W., Omran, H., Deak, E., Henschler, R., Scheele, J.S. & Zieger, B. (2010) Novel integrin-dependent platelet malfunction in siblings with leukocyte adhesion deficiency-III (LAD-III) caused by a point mutation in FERMT3. *Thrombosis and Haemostasis*, **103,** 1053–1064.

Kawai, T. & Malech, H.L. (2009) WHIM syndrome: congenital immune deficiency disease. *Current Opinion in Hematology*, **16,** 20–26.

Khanna-Gupta, A., Sun, H., Zibello, T., Lee, H.M., Dahl, R., Boxer, L.A. & Berliner, N. (2007) Growth factor independence-1 (Gfi-1) plays a role in mediating specific granule deficiency (SGD) in a patient lacking a gene-inactivating mutation in the C/EBPepsilon gene. *Blood*, **109,** 4181–4190.

Kilic, S.S. & Etzioni, A. (2009) The clinical spectrum of leukocyte adhesion deficiency (LAD) III due to defective CalDAG-GEF1. *Journal of Clinical Immunology*, **29,** 117–122.

Kinashi, T., Aker, M., Sokolovsky-Eisenberg, M., Grabovsky, V., Tanaka, C., Shamri, R., Feigelson, S., Etzioni, A. & Alon, R. (2004) LAD-III, a leukocyte adhesion deficiency syndrome associated with defective Rap1 activation and impaired stabilization of integrin bonds. *Blood*, **103,** 1033–1036.

Klein, C. (2009) Congenital neutropenia. *Hematology (American Society of Hematology, Education Program)*, **1,** 344–350.

Klein, C., Grudzien, M., Appaswamy, G., Germeshausen, M., Sandrock, I., Schaffer, A.A., Rathinam, C., Boztug, K., Schwinzer, B., Rezaei, N., Bohn, G., Melin, M., Carlsson, G., Fadeel, B., Dahl, N., Palmblad, J., Henter, J.I., Zeidler, C., Grimbacher, B. & Welte, K. (2007) HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). *Nature Genetics*, **39,** 86–92.

Kollner, I., Sodeik, B., Schreek, S., Heyn, H., von Neuhoff, N., Germeshausen, M., Zeidler, C., Kruger, M., Schlegelberger, B., Welte, K. & Beger, C. (2006) Mutations in neutrophil elastase causing congenital neutropenia lead to cytoplasmic protein accumulation and induction of the unfolded protein response. *Blood*, **108,** 493–500.

Kostmann, R. (1956) Infantile genetic agranulocytosis; agranulocytosis infantilis hereditaria. *Acta Paediatrica. Supplement*, **45,** 1–78.

Ku, C.L., Yang, K., Bustamante, J., Puel, A., von Bernuth, H., Santos, O.F., Lawrence, T., Chang, H.H., Al Mousa, H., Picard, C. & Casanova, J.L. (2005) Inherited disorders of human Toll-like receptor signaling: immunological implications. *Immunological Reviews*, **203,** 10–20.

Ku, C.L., von Bernuth, H., Picard, C., Zhang, S.Y., Chang, H.H., Yang, K., Chrabieh, M., Issekutz, A.C., Cunningham, C.K., Gallin, J., Holland, S.M., Roifman, C., Ehl, S., Smart, J., Tang, M., Barrat, F.J., Levy, O., McDonald, D., Day-Good, N.K., Miller, R., Takada, H., Hara, T., Al Hajjar, S., Al Ghonaium, A., Speert, D., Sanlaville, D., Li, X., Geissmann, F., Vivier, E., Marodi, L., Garty, B.Z., Chapel, H., Rodriguez-Gallego, C., Bossuyt, X., Abel, L., Puel, A. & Casanova, J.L. (2007) Selective predisposition to bacterial infections in IRAK-4-deficient children: IRAK-4-dependent TLRs are otherwise redundant in protective immunity. *The Journal of Experimental Medicine*, **204,** 2407–2422.

Kuijpers, T.W., Van Lier, R.A., Hamann, D., de Boer, M., Thung, L.Y., Weening, R.S., Verhoeven, A.J. & Roos, D. (1997) Leukocyte adhesion deficiency type 1 (LAD-1)/variant. A novel immunodeficiency syndrome characterized by dysfunctional beta2 integrins. *Journal of Clinical Investigation*, **100,** 1725–1733.

Kuijpers, T.W., van Bruggen, R., Kamerbeek, N., Tool, A.T., Hicsonmez, G., Gurgey, A., Karow, A., Verhoeven, A.J., Seeger, K., Sanal, O., Niemeyer, C. & Roos, D. (2007) Natural history and early diagnosis of LAD-1/variant syndrome. *Blood*, **109,** 3529–3537.

Kuijpers, T.W., van de Vijver, E., Weterman, M.A., de Boer, M., Tool, A.T., van den Berg, T.K., Moser, M., Jakobs, M.E., Seeger, K., Sanal, O., Unal, S., Cetin, M., Roos, D., Verhoeven, A.J. & Baas, F. (2009) LAD-1/variant syndrome is caused by mutations in FERMT3. *Blood*, **113,** 4740–4746.

Lagane, B., Chow, K.Y., Balabanian, K., Levoye, A., Harriague, J., Planchenault, T., Baleux, F., Gunera-Saad, N., Arenzana-Seisdedos, F. & Bachelerie, F. (2008) CXCR4 dimerization and beta-arrestin-

mediated signaling account for the enhanced chemotaxis to CXCL12 in WHIM syndrome. *Blood*, **112,** 34–44.

Lanza, F. (1998) Clinical manifestation of myeloperoxidase deficiency. *Journal of Molecular Medicine*, **76,** 676–681.

Lekstrom-Himes, J. & Xanthopoulos, K.G. (1999) CCAAT/enhancer binding protein epsilon is critical for effective neutrophil-mediated response to inflammatory challenge. *Blood*, **93,** 3096–3105.

Lekstrom-Himes, J.A., Dorman, S.E., Kopar, P., Holland, S.M. & Gallin, J.I. (1999) Neutrophil-specific granule deficiency results from a novel mutation with loss of function of the transcription factor CCAAT/enhancer binding protein epsilon. *Journal of Experimental Medicine*, **189,** 1847–1852.

Li, F.Q. & Horwitz, M. (2001) Characterization of mutant neutrophil elastase in severe congenital neutropenia. *Journal of Biological Chemistry*, **276,** 14230–14241.

Lubke, T., Marquardt, T., von Figura, K. & Korner, C. (1999) A new type of carbohydrate-deficient glycoprotein syndrome due to a decreased import of GDP-fucose into the golgi. *Journal of Biological Chemistry*, **274,** 25986–25989.

Lubke, T., Marquardt, T., Etzioni, A., Hartmann, E., von Figura, K. & Korner, C. (2001) Complementation cloning identifies CDG-IIc, a new type of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency. *Nature Genetics*, **28,** 73–76.

Luhn, K., Wild, M.K., Eckhardt, M., Gerardy-Schahn, R. & Vestweber, D. (2001) The gene defective in leukocyte adhesion deficiency II encodes a putative GDP-fucose transporter. *Nature Genetics*, **28,** 69–72.

Marquardt, T., Brune, T., Luhn, K., Zimmer, K.P., Korner, C., Fabritz, L., van der Werft, N., Vormoor, J., Freeze, H.H., Louwen, F., Biermann, B., Harms, E., von Figura, K., Vestweber, D. & Koch, H.G. (1999a) Leukocyte adhesion deficiency II syndrome, a generalized defect in fucose metabolism. *Journal of Pediatrics*, **134,** 681–688.

Marquardt, T., Luhn, K., Srikrishna, G., Freeze, H.H., Harms, E. & Vestweber, D. (1999b) Correction of leukocyte adhesion deficiency type II with oral fucose. *Blood*, **94,** 3976–3985.

Martin, C., Burdon, P.C., Bridger, G., Gutierrez-Ramos, J.C., Williams, T.J. & Rankin, S.M. (2003) Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity*, **19,** 583–593.

Matute, J.D., Arias, A.A., Wright, N.A., Wrobel, I., Waterhouse, C.C., Li, X.J., Marchal, C.C., Stull, N.D., Lewis, D.B., Steele, M., Kellner, J.D., Yu, W., Meroueh, S.O., Nauseef, W.M. & Dinauer, M.C. (2009) A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40 phox and selective defects in neutrophil NADPH oxidase activity. *Blood*, **114,** 3309–3315.

McDowall, A., Inwald, D., Leitinger, B., Jones, A., Liesner, R., Klein, N. & Hogg, N. (2003) A novel form of integrin dysfunction involving beta1, beta2, and beta3 integrins. *Journal of Clinical Investigation*, **111,** 51–60.

McKercher, S.R., Torbett, B.E., Anderson, K.L., Henkel, G.W., Vestal, D.J., Baribault, H., Klemsz, M., Feeney, A.J., Wu, G.E., Paige, C.J. & Maki, R.A. (1996) Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO Journal*, **15,** 5647–5658.

Medvedev, A.E., Lentschat, A., Kuhns, D.B., Blanco, J.C., Salkowski, C., Zhang, S., Arditi, M., Gallin, J.I. & Vogel, S.N. (2003) Distinct mutations in IRAK-4 confer hyporesponsiveness to lipopolysaccharide and interleukin-1 in a patient with recurrent bacterial infections. *The Journal of Experimental Medicine*, **198,** 521–531.

Meeths, M., Bryceson, Y.T., Rudd, E., Zheng, C., Wood, S.M., Ramme, K., Beutel, K., Hasle, H., Heilmann, C., Hultenby, K., Ljunggren, H.G., Fadeel, B., Nordenskjold, M. & Henter, J.I. (2009) Clinical presentation of Griscelli syndrome type 2 and spectrum of RAB27A mutations. *Pediatric Blood & Cancer*, **54,** 563–572.

Melin, M., Entesarian, M., Carlsson, G., Garwicz, D., Klein, C., Fadeel, B., Nordenskjold, M., Palmblad, J., Henter, J.I. & Dahl, N. (2007) Assignment of the gene locus for severe congenital neutropenia to chromosome 1q22 in the original Kostmann family from Northern Sweden. *Biochemical and Biophysical Research Communications*, **353,** 571–575.

Moisoi, N., Klupsch, K., Fedele, V., East, P., Sharma, S., Renton, A., Plun-Favreau, H., Edwards, R.E., Teismann, P., Esposti, M.D., Morrison, A.D., Wood, N.W., Downward, J. & Martins, L.M. (2009) Mitochondrial dysfunction triggered by loss of HtrA2 results in the activation of a brain-specific transcriptional stress response. *Cell Death and Differentiation*, **16,** 449–464.

Mory, A., Feigelson, S.W., Yarali, N., Kilic, S.S., Bayhan, G.I., Gershoni-Baruch, R., Etzioni, A. & Alon, R. (2008) Kindlin-3: a new gene involved in the pathogenesis of LAD-III. *Blood*, **112,** 2591.

Moulding, D.A., Blundell, M.P., Spiller, D.G., White, M.R., Cory, G.O., Calle, Y., Kempski, H., Sinclair, J., Ancliff, P.J., Kinnon, C., Jones, G.E. & Thrasher, A.J. (2007) Unregulated actin polymerization by WASp causes defects of mitosis and cytokinesis in X-linked neutropenia. *The Journal of Experimental Medicine*, **204,** 2213–2224.

Nathan, C. (2006) Neutrophils and immunity: challenges and opportunities. *Nature Reviews. Immunology*, **6,** 173–182.

Ott, M.G., Schmidt, M., Schwarzwaelder, K., Stein, S., Siler, U., Koehl, U., Glimm, H., Kuhlcke, K., Schilz, A., Kunkel, H., Naundorf, S., Brinkmann, A., Deichmann, A., Fischer, M., Ball, C., Pilz, I., Dunbar, C., Du, Y., Jenkins, N.A., Copeland, N.G., Luthi, U., Hassan, M., Thrasher, A.J., Hoelzer, D., von Kalle, C., Seger, R. & Grez, M. (2006) Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nature Medicine*, **12,** 401–409.

Parkos, C.A., Dinauer, M.C., Jesaitis, A.J., Orkin, S.H. & Curnutte, J.T. (1989) Absence of both the 91kD and 22kD subunits of human neutrophil cytochrome b in two genetic forms of chronic granulomatous disease. *Blood*, **73,** 1416–1420.

Pasvolsky, R., Feigelson, S.W., Kilic, S.S., Simon, A.J., Tal-Lapidot, G., Grabovsky, V., Crittenden, J.R., Amariglio, N., Safran, M., Graybiel, A.M., Rechavi, G., Ben-Dor, S., Etzioni, A. & Alon, R. (2007) A LAD-III syndrome is associated with defective expression of the Rap-1 activator CalDAG-GEFI in lymphocytes, neutrophils, and platelets. *Journal of Experimental Medicine*, **204,** 1571–1582.

Person, R.E., Li, F.Q., Duan, Z., Benson, K.F., Wechsler, J., Papadaki, H.A., Eliopoulos, G., Kaufman, C., Bertolone, S.J., Nakamoto, B., Papayannopoulou, T., Grimes, H.L. & Horwitz, M. (2003) Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2. *Nature Genetics*, **34,** 308–312.

Picard, C., Puel, A., Bonnet, M., Ku, C.L., Bustamante, J., Yang, K., Soudais, C., Dupuis, S., Feinberg, J., Fieschi, C., Elbim, C., Hitchcock, R., Lammas, D., Davies, G., Al Ghonaium, A., Al Rayes, H., Al Jumaah, S., Al Hajjar, S., Al Mohsen, I.Z., Frayha, H.H., Rucker, R., Hawn, T.R., Aderem, A., Tufenkeji, H., Haraguchi, S., Day, N.K., Good, R.A., Gougerot-Pocidalo, M.A., Ozinsky, A. & Casanova, J.L. (2003) Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science*, **299,** 2076–2079.

Qasim, W., Cavazzana-Calvo, M., Davies, E.G., Davis, J., Duval, M., Eames, G., Farinha, N., Filopovich, A., Fischer, A., Friedrich, W., Gennery, A., Heilmann, C., Landais, P., Horwitz, M., Porta, F., Sedlacek, P., Seger, R., Slatter, M., Teague, L., Eapen, M. & Veys, P. (2009) Allogeneic hematopoietic stem-cell transplantation for leukocyte adhesion deficiency. *Pediatrics*, **123,** 836–840.

Ron, D. & Walter, P. (2007) Signal integration in the endoplasmic reticulum unfolded protein response. *Nature Reviews. Molecular Cell Biology*, **8,** 519–529.

Rosenberg, P.S., Alter, B.P., Bolyard, A.A., Bonilla, M.A., Boxer, L.A., Cham, B., Fier, C., Freedman, M., Kannourakis, G., Kinsey, S., Schwinzer, B., Zeidler, C., Welte, K. & Dale, D.C. (2006) The incidence of leukemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy. *Blood*, **107,** 4628–4635.

Rosenberg, P.S., Zeidler, C., Bolyard, A.A., Alter, B.P., Bonilla, M.A., Boxer, L.A., Dror, Y., Kinsey, S., Link, D.C., Newburger, P.E., Shimamura, A., Welte, K. & Dale, D.C. (2010) Stable long-term risk of leukaemia in patients with severe congenital neutropenia maintained on G-CSF therapy. *British Journal of Haematology*, **150,** 196–199.

Sabnis, H., Kirpalani, A., Horan, J., McDowall, A., Svensson, L., Cooley, A., Merck, T., Jobe, S., Hogg, N. & Briones, M. (2010) Leukocyte adhesion deficiency-III in an African-American patient. *Pediatric Blood & Cancer*, **55,** 180–182.

Segal, A.W. (2005) How neutrophils kill microbes. *Annual Review of Immunology*, **23,** 197–223.

Segal, A.W. & Abo, A. (1993) The biochemical basis of the NADPH oxidase of phagocytes. *Trends in Biochemical Sciences*, **18,** 43–47.

Singh, A., Zarember, K.A., Kuhns, D.B. & Gallin, J.I. (2009) Impaired priming and activation of the neutrophil NADPH oxidase in patients with IRAK4 or NEMO deficiency. *Journal of Immunology*, **182,** 6410–6417.

Skokowa, J. & Welte, K. (2009) Dysregulation of myeloid-specific transcription factors in congenital neutropenia. *Annals of the New York Academy of Sciences*, **1176,** 94–100.

Skokowa, J., Cario, G., Uenalan, M., Schambach, A., Germeshausen, M., Battmer, K., Zeidler, C., Lehmann, U., Eder, M., Baum, C., Grosschedl, R., Stanulla, M., Scherr, M. & Welte, K. (2006) LEF-1 is crucial for neutrophil granulocytopoiesis and its expression is severely reduced in congenital neutropenia. *Nature Medicine*, **12,** 1191–1197.

Skokowa, J., Fobiwe, J.P., Dan, L., Thakur, B.K. & Welte, K. (2009) Neutrophil elastase is severely down-regulated in severe congenital neutropenia independent of ELA2 or HAX1 mutations but dependent on LEF-1. *Blood*, **114,** 3044–3051.

Smith, M.L., Cavenagh, J.D., Lister, T.A. & Fitzgibbon, J. (2004) Mutation of CEBPA in familial acute myeloid leukemia. *New England Journal of Medicine*, **351,** 2403–2407.

Smith, B.N., Ancliff, P.J., Pizzey, A., Khwaja, A., Linch, D.C. & Gale, R.E. (2009) Homozygous HAX1 mutations in severe congenital neutropenia patients with sporadic disease: a novel mutation in two unrelated British kindreds. *British Journal of Haematology*, **144,** 762–770.

Solanilla, A., Dechanet, J., El Andaloussi, A., Dupouy, M., Godard, F., Chabrol, J., Charbord, P., Reiffers, J., Nurden, A.T., Weksler, B., Moreau, J.F. & Ripoche, J. (2000) CD40-ligand stimulates myelopoiesis by regulating flt3-ligand and thrombopoietin production in bone marrow stromal cells. *Blood*, **95,** 3758–3764.

Stein, S., Ott, M.G., Schultze-Strasser, S., Jauch, A., Burwinkel, B., Kinner, A., Schmidt, M., Kramer, A., Schwable, J., Glimm, H., Koehl, U., Preiss, C., Ball, C., Martin, H., Gohring, G., Schwarzwaelder, K., Hofmann, W.K., Karakaya, K., Tchatchou, S., Yang, R., Reinecke, P., Kuhlcke, K., Schlegelberger, B., Thrasher, A.J., Hoelzer, D., Seger, R., von Kalle, C. & Grez, M. (2010) Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. *Nature Medicine*, **16,** 198–204.

Svensson, L., Howarth, K., McDowall, A., Patzak, I., Evans, R., Ussar, S., Moser, M., Metin, A., Fried, M., Tomlinson, I. & Hogg, N. (2009) Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. *Nature Medicine*, **15,** 306–312.

Tassone, L., Notarangelo, L.D., Bonomi, V., Savoldi, G., Sensi, A., Soresina, A., Smith, C.I., Porta, F., Plebani, A. & Badolato, R. (2009) Clinical and genetic diagnosis of warts, hypogammaglobulinemia, infections, and myelokathexis syndrome in 10 patients. *Journal of Allergy and Clinical Immunology*, **123,** 1170–1173.

Thrasher, A.J. & Burns, S.O. (2010) WASP: a key immunological multitasker. *Nature Reviews. Immunology*, **10,** 182–192.

Tidow, N., Pilz, C., Kasper, B. & Welte, K. (1997) Frequency of point mutations in the gene for the G-CSF receptor in patients with chronic neutropenia undergoing G-CSF therapy. *Stem Cells*, **15**(Suppl 1)**,** 113–119; discussion 120.

Tsai, Y.C., Lee, W.I., Huang, J.L., Hung, I.J., Jaing, T.H., Yao, T.C., Chen, M.T. & Kuo, M.L. (2008) Neutrophil function and molecular analysis in severe leukocyte adhesion deficiency type I without separation delay of the umbilical cord. *Pediatric Allergy and Immunology*, **19,** 25–32.

Uzel, G., Tng, E., Rosenzweig, S.D., Hsu, A.P., Shaw, J.M., Horwitz, M.E., Linton, G.F., Anderson, S.M., Kirby, M.R., Oliveira, J.B., Brown, M.R., Fleisher, T.A., Law, S.K. & Holland, S.M. (2008) Reversion mutations in patients with leukocyte adhesion deficiency type-1 (LAD-1). *Blood*, **111,** 209–218.

Verbeek, W., Wachter, M., Lekstrom-Himes, J. & Koeffler, H.P. (2001) C/EBPepsilon -/- mice: increased rate of myeloid proliferation and apoptosis. *Leukemia*, **15,** 103–111.

Welte, K., Zeidler, C. & Dale, D.C. (2006) Severe congenital neutropenia. *Seminars in Hematology*, **43,** 189–195.

Williams, D.A., Tao, W., Yang, F., Kim, C., Gu, Y., Mansfield, P., Levine, J.E., Petryniak, B., Derrow, C.W., Harris, C., Jia, B., Zheng, Y., Ambruso, D.R., Lowe, J.B., Atkinson, S.J., Dinauer, M.C. & Boxer, L. (2000) Dominant negative mutation of the hematopoietic-specific Rho GTPase, Rac2, is associated with a human phagocyte immunodeficiency. *Blood*, **96,** 1646–1654.

Winkelstein, J.A., Marino, M.C., Johnston, Jr, R.B., Boyle, J., Curnutte, J., Gallin, J.I., Malech, H.L., Holland, S.M., Ochs, H., Quie, P., Buckley, R.H., Foster, C.B., Chanock, S.J. & Dickler, H. (2000) Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine (Baltimore)*, **79,** 155–169.

Xia, J., Bolyard, A.A., Rodger, E., Stein, S., Aprikyan, A.A., Dale, D.C. & Link, D.C. (2009) Prevalence of mutations in ELANE, GFI1, HAX1, SBDS, WAS and G6PC3 in patients with severe congenital neutropenia. *British Journal of Haematology*, **147,** 535–542.

Yamanaka, R., Barlow, C., Lekstrom-Himes, J., Castilla, L.H., Liu, P.P., Eckhaus, M., Decker, T., Wynshaw-Boris, A. & Xanthopoulos, K.G. (1997) Impaired granulopoiesis, myelodysplasia, and early lethality in CCAAT/enhancer binding protein epsilon-deficient mice.

Proceedings of the National Academy of Sciences of the United States of America, **94,** 13187–13192.

Zeidler, C., Germeshausen, M., Klein, C. & Welte, K. (2009) Clinical implications of ELA2-, HAX1-, and G-CSF-receptor (CSF3R) mutations in severe congenital neutropenia. *British Journal of Haematology*, **144,** 459–467.

Zhang, D.E., Zhang, P., Wang, N.D., Hetherington, C.J., Darlington, G.J. & Tenen, D.G. (1997) Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, **94,** 569–574.

Zhang, J., Shehabeldin, A., da Cruz, L.A., Butler, J., Somani, A.K., McGavin, M., Kozieradzki, I., dos Santos, A.O., Nagy, A., Grinstein, S., Penninger, J.M. & Siminovitch, K.A. (1999) Antigen receptor-induced activation and cytoskeletal rearrangement are impaired in Wiskott–Aldrich syndrome protein-deficient lymphocytes. *The Journal of Experimental Medicine*, **190,** 1329–1342.

Zhang, H., Schaff, U.Y., Green, C.E., Chen, H., Sarantos, M.R., Hu, Y., Wara, D., Simon, S.I. & Lowell, C.A. (2006) Impaired integrin-dependent function in Wiskott–Aldrich syndrome protein-deficient murine and human neutrophils. *Immunity*, **25,** 285–295.

Zhuang, D., Qiu, Y., Kogan, S.C. & Dong, F. (2006) Increased CCAAT enhancer-binding protein epsilon (C/EBPepsilon) expression and premature apoptosis in myeloid cells expressing Gfi-1 N382S mutant associated with severe congenital neutropenia. *Journal of Biological Chemistry*, **281,** 10745–10751.

Zicha, D., Allen, W.E., Brickell, P.M., Kinnon, C., Dunn, G.A., Jones, G.E. & Thrasher, A.J. (1998) Chemotaxis of macrophages is abolished in the Wiskott–Aldrich syndrome. *British Journal of Haematology*, **101,** 659–665.