Congenital neutropenia: advances in diagnosis and treatment

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Purpose of review

A decade after the availability of hematopoietic growth factors, the long-term outcome of severe congenital neutropenia has dramatically changed. The prolonged survival of neutropenic patients receiving hematopoietic growth factors has drawn attention to the heterogeneity of this disease and to the complications of treatment. The dose of granulocyte colony stimulating factor that is required to obtain normal levels of circulating neutrophils and to prevent fever and infections is quite variable among patients, but is higher in children with severe congenital neutropenia than in those with other conditions of neutropenia. Moreover, leukemic transformation during treatment is not observed in all patients, but is more typical of severe congenital neutropenia and Shwachman–Diamond patients.

Recent findings

In recent years, the converging efforts of hematologists, immunologists and geneticists have led to the discovery of the genetic and biochemical basis of severe congenital neutropenia; cyclic neutropenia; warts, hypogammaglobulinemia, immunodeficiency, myelokathexis or WHIM syndrome and other rarer conditions associated to neutropenia.

Summary

Although the diagnosis of congenital neutropenia includes many disorders of distinct origin and variable prognosis, their treatment is still based on granulocyte colony stimulating factor administration. Understanding the pathogenesis of these forms of neutropenia and their evolution will focus future studies on the mechanisms of normal and pathological myelopoiesis and on the development of the most appropriate treatment for each type of neutropenia.

Keywords

AP3 complex, Barth syndrome, cyclic neutropenia, leukocyte elastase, severe congenital neutropenia, WHIM syndrome

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Abbreviations

AIN	autoimmune neutropenia
AML	acute myeloblastic leukemia
ANC	absolute neutrophil count
BMT	bone marrow transplantation
G-CSF	granulocyte colony stimulating factor
HSCT	hematopoietic stem cells transplantation
MDS	myelodysplastic syndrome
SCN	severe chronic neutropenia
SDF1α	stromal-derived factor 1 α
SDS	Shwachman–Diamond syndrome
WHIM	warts, hypogammaglobulinemia, immunodeficiency, myelokathexis

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Introduction

Severe neutropenia constitutes a heterogeneous group of genetic and acquired hematopoietic disorders that are characterized by a reduction of the absolute neutrophil counts (ANCs) that persist for several months and predispose patients to bacterial infections, including deep tissue infections, sepsis, and fever [1,2]. There is a great degree of variability in the lower limit of neutrophil blood counts that is both age- and race-dependent because normal levels are lower in infants (1×10^9/l) than in older children and adults (1.5×10^9/l) and in black than in Caucasian peoples. However, recurrent bacterial infections are not frequently observed until ANCs fall below the limit of severe neutropenia (cells $< 0.5 \times 10^9$/l). Even patients with the same number of blood circulating neutrophils may display various degrees of susceptibility to bacterial infections because the causative mechanism influences the clinical outcome of the disease. As a matter of fact, disorders of myelopoiesis such as severe chronic neutropenia (SCN) are characterized by a larger number and severity of infectious episodes than acquired neutropenia types such as autoimmune neutropenia, which usually lacks major sepsis episodes.

Autoimmune neutropenia

Destruction of neutrophils because of antibodies directed against them results in autoimmune neutropenia (AIN). This acquired form of neutropenia constitutes the most common cause of selective defects of neutrophil generation, at least 10-fold more common than SCN (incidence 1:100 000), but is often not identified because detection of granulocyte autoantibodies and alloantibodies has proven to be troublesome and uncertain in many cases [3–6]. It usually presents in the first 2–3 years of life with minor bacterial infections of the skin and of the ear, but sometimes the disease is asymptomatic and is identified on the basis of a blood work

performed for other reasons. Primary autoimmune neutropenia should be differentiated from the neonatal allo-immune neutropenia that is usually recognized at birth or during the first months of life [4,7,8]. This latter condition does not usually require specific therapy despite low neutrophil counts because infants recover spontaneously within 6 months upon disappearance of neutrophil specific alloantibodies from the blood. Diagnosis can be made based on the detection of IgG antibodies directed against granulocyte antigens inherited from the father that are also present in the mother's serum.

For both alloimmune neutropenia and primary autoimmune neutropenia the severity and the number of bacterial infections is much lower than in genetically inherited neutropenia. But, differentiation of these conditions from SCN is not always straightforward because severe infections, that can occur in AIN, may result in hypocellular bone marrow or in maturation arrest at the myelocyte/metamyelocyte stage, thereby mimicking the other more severe diseases [3]. In addition, detection of granulocyte specific autoantibodies is not always possible because of the low sensitivity and specificity of the assays. Most of the autoantibodies identified are directed against common cell surface antigens of neutrophils such as CD11b/CD18, FcγRIIIb and FcγRII, but their identification is not easy because these receptors are able to spontaneously bind immunoglobulins thereby hampering the sensitivity of the assay [3].

Differential diagnosis of SCN and AIN should also be based on clinical considerations such as the spontaneous remission of AIN without requirement of granulocyte colony stimulating factor (G-CSF) treatment. The use of antibiotics and of hematopoietic factors should be limited to infections that require hospital admission or before surgical intervention. The benign course of AIN is probably related to the normal myelopoietic activity of bone marrow that is sufficient to release an increased number of neutrophils during infections because of the endogenous secretion of G-CSF.

Myelokathexis

This condition identifies the retention of mature neutrophils in bone marrow despite neutropenia in peripheral blood. Association of myelokathexis with hypogammaglobulinemia, leucopenia and warts was first described by Zuelzer in 1965 as warts, hypogammaglobulinemia, immunodeficiency, myelokathexis (WHIM) syndrome, usually inherited as an autosomal dominant disease [9]. Although verrucosis and hypogammaglobulinemia are not observed in all the cases, neutropenia and a hypercellular bone marrow are typically observed. Myeloid and lymphoid cells are normally generated in bone marrow but are not released to the bloodstream resulting in a hypercellular bone marrow with hypersegmented and hypermature neutrophils [10]. Neutrophil blood counts are starkly reduced (ANC $< 0.5 \times 10^9$/l), but during infectious episodes their number rises suddenly, thus confirming a normal hematopoietic response to infections.

The disease is characterized by a benign course with recurrent respiratory infections (pneumonia, sinusitis and acute otitis media), although meningitis, cellulitis and sepsis were occasionally described. Besides neutropenia, patients with myelokathexis also present with a moderate reduction of lymphocyte counts, and imbalance in distribution of T and B cell subsets, suggesting an abnormal trafficking of these cell subsets between lymphoid organs [11]. Hernandez *et al.* [12••] recently reported that WHIM syndrome is caused by heterozygous mutations of the chemokine receptor CXCR4 (Table 1). For most of their patients, missense mutations or deletions that result in truncation of the cytoplasmic tail were described, suggesting that these mutations may probably lead to abnormal signaling of the chemokine receptor [12••,13•]. Both neutrophils and lymphocytes of WHIM patients display increased chemotaxis, adhesion and trans-endothelial migration in response to the ligand stromal-derived factor 1 α (SDF1α), or CXCL12, that is expressed by stromal and endothelial cells of bone marrow [11,13•]. Expression of the truncated form of CXCR4 confers increased responsiveness of mature cells to the chemokine and prevents their marginalization to the bone marrow and subsequent release to the bloodstream [11]. During inflammatory conditions, however, leukocytes may be diverted from bone marrow to the circulation because the increased blood levels of the chemokines CXCL8 and CCL2 may counteract the increased functional activity of CXCL12. This model is supported by the evidence that CXCL8 administration to monkeys leads to rapid mobilization of leukocytes and hematopoietic precursors from bone marrow to the bloodstream [14]. Moreover, administra-

Table 1. Genetic diagnosis of inherited neutropenia

Disease	Inheritance	OMIM	Gene
Myelokathexis (WHIM)	AD	#193670	*CXCR4*
Severe congenital neutropenia	AD	#202700	*ELA2*
Severe congenital neutropenia	AD	#202700	*Gfi1*
Cyclic neutropenia	AD	#162800	*ELA2*
Hermansky–Pudlak syndrome 2	AR	#608233	*AP3B1*
Shwachman–Diamond syndrome	AR	#260400	*SBDS*
Barth syndrome	X-linked	#302060	*TAZ*
X-linked congenital neutropenia	X-linked	#300299	*WASP*
Glycogen storage disease 1b	AR	#232220	*G6PT1*
Hyper-IgM 1	X-linked	#308230	*TNFSF5*
Hyper-IgM 3	AR	#606843	*TNFRSF5*

WHIM, warts, hypogammaglobulinemia, immunodeficiency, myelokathexis; AR, autosomal recessive; AD, autosomal dominant.

tion of the CXCR4 antagonist AMD-3100 to healthy subjects induces mobilization of leukocytes and increases the effect of G-CSF, suggesting that myelokathexis occurs because of disturbance of the homeostatic distribution of leukocytes between bone marrow and blood [15•].

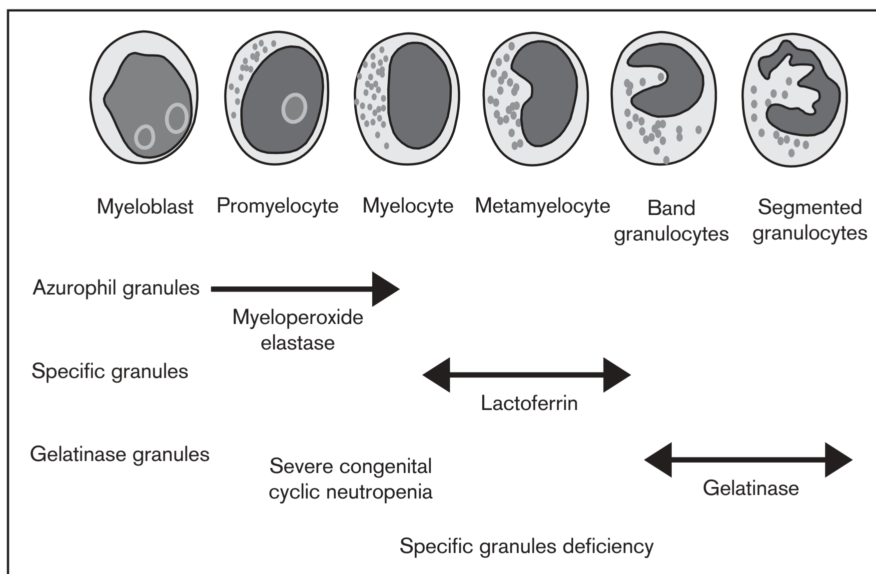
Severe congenital neutropenia

Congenital neutropenia was first described by Kostmann in 1956 in a large intermarried Swedish family as infantile agranulocytosis with autosomal recessive inheritance [2]. Thereafter all the attempts to identify the causes of inherited selective cytopenia of the myeloid lineage were unsuccessful for many years. More recent studies have demonstrated that the most common cause of genetic neutropenia, SCN, is inherited as an autosomal dominant trait with an estimated frequency of one to two cases per million, and is caused by mutations in the leukocyte elastase gene, *ELA2* (Table 1). The diagnosis of SCN is usually made on the basis of the observation at birth, or in the following months of life, of a severe reduction of ANC ($<0.5\times10^9$/l, but most often ANCs are less than 0.2×10^9/l) with normal or near normal levels of hemoglobin and platelets, while bone marrow examination reveals the typical defect of neutrophils with myeloid cell differentiation arrest at the promyelocyte stage and very few myelocytes and metamyelocytes are present [16]. Identification of heterozygous mutations of *ELA2* as the most common cause of SCN was recently reported. This conclusion was based on positional cloning after demonstration that both loci for autosomal dominant cyclic neutropenia and for SCN map to chromosome 19p13.3, in a region where genes encoding several serine proteases are located, including leukocyte elastase, proteinase 3 and the protease homologue azurocidin [17,18]. Mutational analysis in families and in sporadic cases of SCN have demonstrated that most of the patients with the disease display mutations of *ELA2* that segregate with the disease in all affected members of families [17–19]. Because of the autosomal dominant mechanism of inheritance, numerous hypotheses about its pathogenesis have been suggested. Haploinsufficiency appears an improbable justification for the role of *ELA2* mutations in the pathogenesis of neutropenia, because null mice transfected with heterozygous or homozygous mutants of the enzyme do not display alteration in the differentiation of myeloid cells [20]. Moreover, missense and deletion mutations of *ELA2* do not necessarily lead to loss or reduction of serine protease enzymatic activity, but result in conformational changes that probably exert a dominant negative effect on myeloid differentiation [21]. An alternative explanation to the neutropenia may reside in a toxic gain of function and loss of inhibition by the natural inhibitor α_1-antitrypsin [17]. Leukocyte elastase belongs to the family of hematopoietic serine protease and is a 27–31 kDa glycoprotein that is expressed by myeloid cells, including monocytes and neutrophils, where it is stored in primary granules [22]. Since leukocyte elastase is synthesized in a preproprotease form of inactive enzyme, it has been postulated that mutant enzymes might be released in an enzymatically active form from the endoplasmic reticulum of myeloid cells, thereby leading to cell death. This hypothesis is supported by studies showing both microscopic and functional evidence of accelerated apoptosis of myeloid progenitors in the bone marrow of neutropenic patients [23–25]. However, in-vitro expression of mutant forms of the enzyme that are associated with SCN retain vulnerability to inhibition by α_1-antitrypsin and fail to demonstrate any toxic activity for cells, thus suggesting that arrest of myelopoiesis does not arise from a toxic activity of the enzyme [21].

Leukocyte elastase is transiently expressed in the promyelocyte stage of myeloid differentiation, is packaged with other serine proteases (cathepsin-G, PR3) and antibacterial proteins (myeloperoxidase, azurocidin) and localizes in neutrophil primary granules. Although leukocyte elastase is not selectively expressed by neutrophils and is detectable in other cells of myeloid origin, the process of coordinated expression of the protein components that participate in generation of primary granules from the Golgi network is selectively activated in granulocytes (Fig. 1) [22]. Leukocyte elastase is synthesized in the endoplasmic reticulum in an inactive form that is composed of the signal peptide, the N-terminal dipeptide, the central mature protein and a C-terminal extension prodomanin of 20 residues (248–267). While the N-terminal dipeptide is required to preserve the proenzyme in an inactive form before it is transported to the granular compartment, the C-terminal extension does not affect the proteolytic activity of the protein, but confers a signal that inhibits transportation of the inactive form of the preproelastase to granules before the post-translational maturation of the protein is completed [22,26,27•,28]. Correct localization of elastase to primary granules requires interaction of its processed form with the adaptor protein complex AP3. This cytosolic tetrameric complex functions to shuttle transmembrane cargo proteins from the trans-Golgi network to the lysosomes. Although elastase is generally a soluble protein, there is evidence that leukocyte elastase is also expressed in an intermediate stage as a disulfide bonded transmembrane protein that extrudes its C-terminal tail through the lipid bilayer [27•]. Upon enzymatic removal of the C-terminal extension, the protein maintains a transmembrane conformation capable of associating with AP3 complexes. Interaction of cytosolic AP3 with membrane-associated elastase requires the tyrosine-based sorting signal that is located in the carboxyl-terminal domain of the protein. It is interesting to point out that most mutations in *ELA2*

Figure 1. Stages of neutrophil granulocyte maturation

Granule formation is associated with sequential expression of enzymes and antibacterial proteins that are transiently expressed during neutrophil differentiation. Block in this expression program results in neutropenia or in neutrophil dysfunction.



associated with SCN result in removal of the sorting signal, thereby favoring the mislocation of the enzyme to the membrane [27]. Conversely, normal interaction of elastase with AP3 complex allows its correct shuttling and release in a soluble form in the lumen of primary granules. Horwitz *et al.* have proposed that the localization of the enzyme in the lumen of granules or on the plasma membrane may regulate the differentiation pattern of myeloid progenitor cells into monocytes or granulocytes suggesting that neutropenia may result from a defective differentiation switch between the monocytic and granulocytic lineage of myelopoiesis [28,29]. This attractive hypothesis may account for the observation that granulocytes and monocytes reciprocally cycle in normal hematopoiesis and for the typical increase of monocyte blood counts observed in the majority of SCN patients. Recent data indicate that elastase might regulate the fate of myeloid progenitor cells by interfering with signaling regulators of hematopoiesis known as Notch proteins [30•]. Members of the Notch family are capable of interacting with transcription factors, such as PU.1, that are expressed during myeloid development, thereby switching monocytic differentiation to the granulocytic pathway [31,32].

Severe congenital neutropenia has also been observed in four patients (three belonging to the same family), with heterozygous mutations of the transcriptional repressor oncoprotein *Gfi1* (Table 1) [33]. Segregation of the disease was suggestive of an autosomal dominant pattern of transmission. Neutrophil counts were quite variable among subjects varying from zero in a 4-month-old boy up to 0.9×10^9/l in his 3-year-old brother, but T lymphocytes, especially naïve T cells, and B lymphocytes also were markedly reduced. The combination of leukopenia and immunodeficiency is reminiscent of WHIM syndrome, but, in these patients, the bone marrow was populated by immature myeloid cells instead of hypermature neutrophils [10].

Both mutations of *Gfi1* were amino acid substitutions in one of the zinc finger domains that interfere with the transcriptional repressor activity by a dominant negative mechanism. Moreover, the authors observed that several Gfi1 binding motifs are present in the *ELA2* promoter region, and that leukocyte elastase activity is increased in cells of neutropenic patients [33]. These observations suggest that Gfi1 is a negative regulator of leukocyte elastase expression and that the heterozygous mutations detected in patients interfere with the repressor activity of the transcription protein. Overall, these observations further underline the crucial role of elastase expression for normal differentiation of myeloid cells.

Idiopathic neutropenia

This is a diagnosis of exclusion that is utilized for neutropenic patients who do not display any known cause of neutropenia. Although the condition is obviously heterogeneous, the majority of patients display a satisfactory response to treatment with G-CSF and have a good prognosis [6].

Cyclic neutropenia

Periodic oscillations of neutrophil counts associated with fever and mouth ulcers were primarily identified as a distinct pathological condition since 1910. In these

patients, episodes of neutropenia ($<0.2\times10^9$/l) recur with the regular period of 21 days, persist for 3–5 days and are characterized by infectious events that are usually less severe than in SCN [16,18,34]. Although the clinical presentation of cyclic neutropenia is sufficiently peculiar to distinguish this condition from SCN, it is now clear that cyclic neutropenia is also caused by heterozygous mutations of *ELA2* (Table 1), the same locus that is associated to SCN [17–19]. However, many mutations associated with cyclic neutropenia are located near the junction between exons 4 and 5, while mutations in SCN patients are primarily in the coding regions of exons 2, 3, 4 and 5 [18]. This hypothetical model of genotype/phenotype correlation based on genetic studies was further clarified by biochemical data showing that mutations of *ELA2* in cyclic neutropenia patients were associated with a different location of the enzyme in neutrophils. Most of the mutations reported in cyclic neutropenia patients lead to loss of C-terminal extension or disrupt the hydrophobic structure of the predicted transmembrane domains, thereby resulting in preferential accumulation of elastase in granules [27•]. Although these observations suggest the molecular mechanism of cyclic neutropenia, its pathogenesis is largely based on theoretical considerations. In particular, it has been proposed that loss of a regulatory feedback loop might result in cyclic hematopoiesis because of the intrinsic periodicity of blood-cell production from the bone marrow.

Chronic congenital neutropenia in association with syndromes and metabolic conditions

Congenital neutropenia is observed in numerous syndromes and metabolic diseases that are characterized by other manifestations (Table 1). Recognition of neutropenia and recurrent infections is important for the identification of these conditions and for their appropriate treatment. Moreover, understanding the pathogenesis of these genetic conditions contributes to highlight the mechanism of normal myelopoiesis and to identify novel potential treatments for neutropenia.

Hermansky–Pudlak syndrome 2

In this disease, neutropenia is associated with oculocutaneous albinism and moderate bleeding disorders [35,36]. Recurrent respiratory infections occur because of severe neutropenia, that is responsive to G-CSF treatment, and of defective cytotoxic activities of CD8+ cells [37,38••]. This autosomal recessive disease is caused by mutations of the gene encoding for the beta3 component of the AP3 complex (Table 1); the lack of one of the subunits that constitute the tetrameric complex of AP3 prevents the shuttle of transmembrane cargo proteins, including leukocyte elastase, from the trans-Golgi network to the lysosomes in hematopoietic cells and in melanocytes [38••,39••]. Study of cyclic neutropenia in dogs has demonstrated that this condition is equivalent to type 2 Hermansky–Pudlak and is characterized by defective formation of elastase-containing granules in myeloid cells of the animals, thereby leading to incomplete differentiation of neutrophils [27•,28].

Shwachman–Diamond syndrome

Leukopenia, exocrine pancreatic insufficiency, skeletal abnormalities, and short stature are the prototypical manifestations of this autosomal recessive disease [40]. It presents most often in infancy with bacterial infections and failure to thrive because of the abnormal function of the gastrointestinal system. The causative gene of the disease was recently identified by positional cloning in families of patients affected by Shwachman–Diamond syndrome (SDS) in a previously uncharacterized gene termed *SDBS* (Table 1), for Shwachman–Bodian–Diamond Syndrome [41•]. Recurrent mutations result from gene conversion in 89% of unrelated individuals with SDS; recombination events occur between *SDBS* and a highly conserved pseudogene without coding potential (*SBDSP*, 97% nucleotide identity) that resides 5.8 Mb distally [41•]. *SBDS* is ubiquitously expressed in both hematopoietic and non-hematopoietic tissues, including leukocytes and exocrine pancreas, and encodes for a previously uncharacterized 28 kDa protein with sequence similarity to RNA binding proteins [41•].

Neutropenia is constantly observed in patients affected by this disease, but neutrophil counts vary, ranging from normal values and falling to low levels in the same subject. However, all the hematopoietic lineages may be affected, as mild anemia and thrombocytopenia are described [40,42,43]. Cytopenias may reflect respective changes of bone marrow cellularity that often involve the myeloid compartment, but various dysplastic variations of the erythroid compartment can be detected, even if circulating erythrocytes are normal. In association with cytopenias and hematopoietic dysplasia, cytogenetic abnormalities, and increased risk of transformation in myelodysplastic syndrome (MDS)/acute myeloblastic leukemia (AML) are often observed in SDS patients [42,44]. Indeed, follow-up studies of SDS patients carrying various degrees of cytogenetic abnormalities demonstrate that these patients may develop myelodysplasia, but some cytogenetic changes may spontaneously regress indicating that risk prediction may be difficult in SDS patients [45,46].

Several authors have reported a defect of mobility and chemotaxis in response to chemoattractants of neutrophils in SDS patients [40,43]. Analysis of neutrophil migration in spatial gradients of chemoattractants has demonstrated that cells from patients display severe defects in orientation and migration of cells, thereby

suggesting that this defect may account for the abnormal hematopoiesis that is observed in SDS patients [47].

Cardioskeletal myopathy (Barth syndrome)

Cardiac dysfunction, usually presenting as dilated cardiomyopathy in the first year of life, but sometimes even in the last trimester of pregnancy as ventricular dilatation, is associated with cyclic neutropenia and severe infections [48••]. Even in the same patient, the extent of neutropenia is variable, changing from near-normal values to severe neutropenia, without any abnormality of neutrophil function, including phagocytosis and killing [49]. Barth syndrome can be suspected when neutropenia is found with muscle weakness or dilated cardiomyopathy, but the diagnosis should be based on biochemical studies of cardiolipin synthesis or on mutagenesis analysis [50]. The causative gene *TAZ* is located on *Xq28* (Table 1) and encodes for an enzyme with acyltransferase activity that is probably required for synthesis of cardiolipin, a component of the inner mitochondrial membrane that is essential for the stability and function of mitochondria [48••,51]. It is interesting that a lack of cardiolipin synthesis confers to the neutrophil plasma membrane of Barth patients an increased binding capacity to annexin V, without evidence of enhanced apoptosis [52•], suggesting that abnormal differentiation of myeloid cells could be related to changes in the biochemical structure of lipid bilayers in neutrophils.

Examination of bone marrow demonstrates a maturation arrest at the promyelocyte stage of myeloid differentiation, reproducing the same hematological defect that is observed in SCN. Like SCN patients, patients with Barth syndrome have been successfully treated with G-CSF [48••,49].

Glycogen storage disease type 1b

Severe neutropenia and defective neutrophil respiratory burst are caused by deficiency in glucose-6 translocase (Table 1), an enzyme that functions to transport glucose-6-phosphate into the cytoplasmic reticulum. Lack of the enzyme results in insufficient gluconeogenesis or glycogenolysis, thereby leading to hypoglycemia and lactic acidosis and requiring dietary carbohydrates [53]. Conceivably, abnormal glucose metabolism in myeloid progenitors may increase susceptibility to apoptosis, thereby leading to generation of dysfunctional neutrophils in circulation [54•]. G-CSF is an effective treatment for patients with the disease inducing an increase of neutrophil counts by reduction of infections and improving the inflammatory bowel disease that is often detected in glycogen storage disease type 1b patients [55,56].

X-linked neutropenia

Wiskott–Aldrich syndrome is an X-linked disorder that is classically identified on the basis of the association of thrombocytopenia with eczema and recurrent infections. The disease is caused by mutation of the gene encoding the adaptor protein, WASp (Table 1), that is involved in regulation of cytoskeleton organization in hematopoietic cells [57]. Complete lack of WASp expression is associated with complete clinical manifestation of the disease, while hypomorphic mutations of the gene are associated with preservation of protein expression resulting in X-linked thrombocytopenia [58]. A particular missense mutation (L270P) that affects the conserved GTPase binding domain of the protein, however, is associated with development of X-linked severe congenital neutropenia with maturation arrest at the promyelocyte–metamyelocyte stage [59]. Although the pathogenic mechanism remains unclear, it is likely that this missense mutation may lead to defective signaling in response to stimuli that regulate cytoskeleton organization [60]. Therefore, it is likely that response to chemokines such as CXCL12/SDF1 may be altered in patients carrying the L270P mutation of *WASp*.

Hyper-IgM

Severe neutropenia has been reported in a large number of patients with X-linked hypogammaglobulinemia due to CD40-ligand (CD40L) deficiency [61]. Interestingly, neutropenia was also observed in one patient with autosomal recessive hyper-IgM due to CD40 deficiency (Table 1), but it is not reported in patients affected by the hyper-IGM type 2 syndrome caused by mutations of the enzyme, activation-induced cytidin deaminase, required for B cell class switch recombination [62,63]. A common interpretation of this finding is that a lack of cognate interaction between activated T cells, expressing CD40L and macrophages/dendritic cells, that express CD40, may result in unbalanced regulation of cytokine secretion and subsequently to autoimmune reaction. Alternatively, CD40 expression by myeloid progenitors may act as an intrinsic regulator of myelopoiesis.

Treatment of neutropenia

The availability of hematopoietic growth factors has dramatically changed the prognosis of congenital and acquired neutropenia [64,65]. Past publications describing congenital neutropenia reported that 42% of patients died at a mean age of 2 years because of sepsis and neutropenia [1]. Over the last decade, the introduction of treatment with G-CSF has reduced the number and the severity of infectious episodes, prolonging the survival and the extent of time during which neutropenic patients remained free of life-threatening infections [16,65]. Previous attempts at treatment with immunouppressive drugs, immunoglobulins and antibiotic prophylaxis have not been generally successful, because the observed benefits were usually transient or were associated with superinfection by resistant bacteria or

fungi [66]. Clinical trials and reports from international registries suggest that the vast majority of neutropenic patients (>90%) respond to treatment with G-CSF at dosages lower than 30 μg/kg with a mean ANC increase of more than $1.5–2.0 \times 10^9$ cells/l [16,65]. A satisfactory response is usually observed within 1–2 weeks and is sustained for at least 6–10 years without loss of responsiveness. Patients with SCN have an heterogeneous response to this therapy and often require a gradual increase of G-CSF dose before the optimal dosage can be found. In addition, some SCN patients do not display an adequate response to treatment. Variability in the individual response to G-CSF reflects distinct pathogenetic conditions, because patients with cyclic neutropenia or idiopathic neutropenia require lower doses of the cytokine (2.4–2.6 μg/kg) than SCN patients (11–13 μg/kg). In addition, 3–5% of SCN patients are refractory to G-CSF treatment, even if large daily doses of the cytokine are used (up to 100 μg/ml) and may experience recurrent episodes of life-threatening infections [16,19,65]. Genetic and hematological characterization of these patients has identified that certain mutations, such as the G185R, are associated with severe promyelocyte–metamyelocyte maturation arrest and to insufficient neutrophil increase upon G-CSF treatment, thus supporting the hypothesis that elastase mutations may lead to loss of responsiveness to G-CSF stimulation of myeloid progenitors [19]. Early description of patients affected by SCN that did not respond to G-CSF therapy pointed out that mutations in the G-CSF receptor (*CSF3R*) could confer a hyporesponsiveness of myeloid cells to the cytokine, thus leading to a maturation arrest of bone marrow progenitor cells at the stage of promyelocyte. However, only a single case of mutation in the extracellular domain of *CSF3R* in a SCN patient has been reported, while all the other neutropenic patients carried mutations which result in truncation of the 82–98 carboxyl-terminal amino acids and confer sustained receptor activation and hyperproliferative response to G-CSF [67]. Investigation of *ELA2* and *CSF3R* genotypes in SCN patients at serial time points has demonstrated that while mutations of *ELA2* are congenital and segregate with the disease in affected members, mutations of *CSF3R* are acquired during the years of follow-up and confer a hematopoietic advantage to stem cells [68]. In addition, *CSF3R* mutations are more frequent in SCN patients that develop MDS or AML, but are not necessarily associated to leukemic transformation [69]. Follow-up studies of neutropenic patients receiving treatment for at least 8 years have demonstrated that the overall risk of disease conversion to MDS/AML ranges from 9 up to 13%, remaining fairly constant (less than 2% per year) during the first years of treatment [16,66]. In addition, these studies have addressed the issue of a causal relationship between the length of G-CSF therapy and the risk of leukemic transformation, without observing a correlation. MDS/AML conversion was observed in SCN and in SDS patients but not in other groups of neutropenic patients including cyclic neutropenia or chronic idiopathic neutropenia [16,19,65]. These observations suggest that the same pathogenetic mechanisms that lead to the maturation arrest at the promyelocyte stage may confer an increased risk of myeloid progenitors to other leukemogenic factors. This hypothesis is supported by evidence that MDS/AML development in SCN patients is preceded by cytogenetic abnormalities, including chromosome 7 monosomy and chromosome 21 alterations, and by activating mutations of both the oncogene *ras* and the cytokine receptor *CSF3R* [65,70•,71]. Although the sequence of the leukemia development is unclear, it is likely that the maturation arrest constitutes the predisposing event that increases the risk that cytogenetic abnormalities and oncogene mutations may activate this program leading to malignant transformation.

Treatment of MDS/AML with chemotherapy or bone marrow transplantation (BMT) is associated with a high mortality rate higher (70%), thus suggesting that an accurate follow-up of SCN is required to detect early cytogenetic and genetic events that lead to conversion of the disease. Annual screening for *CSF3R* mutations and cytogenetic changes should be performed to identify SCN patients who would benefit from BMT or hematopoietic stem cell transplantation. Stem cell transplantation has been performed in SCN patients that were refractory to G-CSF or who developed genetic/cytogenetic transformation of bone marrow that are associated with increased risk of MDS/AML conversion [72]. Of the 11 patients that received BMT/hematopoietic stem cells transplantation (HSCT), eight underwent successful transplantation from human leukocyte antigen-identical donors, two receiving mismatched bone marrow died after transplantation, while the remaining one has lost the initial engraftment and requires treatment with G-CSF [72]. Overall, these results suggest that G-CSF treatment remains a safer option for the vast majority of patients with neutropenia, while BMT/HSCT should be reserved for SCN patients at risk for leukemic transformation. Additional indications for BMT/HCST in SCN patients may be justified by new cell transplantation procedures that are currently under development.

Conclusion

Daily treatment with G-CSF for many genetic and acquired conditions leading to neutropenia has provided a useful tool in the prevention of life-threatening infections in these patients, but has also demonstrated the great degree of heterogeneity in the pathogenesis of these conditions. Precise characterization of the genetic

and hematological basis of severe congenital neutropenia has demonstrated that leukocyte elastase plays a key role in the regulation of granulocytic differentiation, providing the link between signals that arise from the bone marrow environment to transcription regulators that are activated during neutrophil differentiation. Abnormal synthesis of leukocyte elastase and its cytosolic location in primary granules or interaction with signaling proteins of the Notch family, may prevent the switch of myeloid progenitors from the monocytic differentiation to the granulocytic path, thereby leading to maturation arrest at the promyelocyte stage. Several biochemical mechanisms may lead to a maturation arrest of myeloid differentiation in such disparate conditions as Barth syndrome, glycogen storage disease type 1b, hyper IgM, or X-linked neutropenia, resulting in the same clinical and hematological manifestations of SCN. In future years understanding these mechanisms will provide alternative and more appropriate therapeutic options for disorders of myelopoiesis.

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- • of special interest
- •• of outstanding interest

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