Congenital Neutropenia

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Congenital neutropenia syndromes represent a heterogenous group of disorders affecting both neutrophil homeostasis and neutrophil function (Boztug et al., 2008b; Welte et al., 2006). The neutropenia is called „severe“ when absolute neutrophil counts (ANC) are below 0.5x10⁹/L. Patients with severe congenital neutropenia (SCN) suffer from recurrent bacterial and fungal infections, and without treatment with recombinant human G-CSF (rh-GCSF) children usually die within their first years of life (Bonilla et al., 1989; Welte et al., 1990), while treatment with rh-G-CSF can lead to increased ANCs and decreased infectious complications. However, the prognosis of patients with SCN is limited by a persistent susceptibility to infections even with normalized neutrophil counts and the evolution of myelodysplastic syndromes or acute myeloid leukemia (AML). At present, the only curative therapy consists of allogeneic bone marrow transplantation, which is associated with a favorable prognosis only when an HLA-matched donor is available (Choi et al., 2005; Ferry et al., 2005; Zeidler et al., 2000).

Genetics of congenital neutropenia syndromes

Remarkable progress has been made with regards to the identification of the genetic defects causing SCN. The disease was initially described by the Swedish pediatrician Rolf Kostmann in 1956 (Kostmann, 1956). Horwitz et al. discovered heterozygous mutations in the gene encoding neutrophil elastase (ELA2) in patients with autosomal dominant cyclic neutropenia (CyN), a condition with oscillating neutrophil counts but less severe clinical symptoms (Horwitz et al., 1999). Subsequently, it was recognized that many patients suffering from autosomal dominant or sporadic SCN also carry heterozygous mutations in ELA2 (Dale et al., 2000). To date, more than 50 distinct ELA2 mutations have been described in SCN (Horwitz et al., 2007), accounting for approximately 40% of patients with SCN (SCN international registry, unpublished results, 2008). We have recently been able to identify mutations in the gene encoding the mitochondrial protein HAX1 as a cause of autosomal recessive SCN (Klein et al., 2007). HAX1 mutations were also identified as the cause of SCN in the original Kostmann pedigree (Klein et al., 2007). HAX1 mutations may account for approximately 15% of patients with SCN (SCNIR, unpublished results). Very rare genetic causes of SCN comprise mutations in GFI (Person et al., 2003), which is a zink-finger transcription factor critical for myeloid differentiation, or activating mutations in the WAS gene which disrupt the autoinhibitory state of the protein (Ancliff et al., 2006; Devriendt et al., 2001). Recently, we were able to discover a novel subtype of SCN associating neutropenia and complex developmental aberrations, caused by mutations in the glucose-regulating gene G6PC3, the gene product of which is located in the endoplasmic reticulum (Boztug et al., 2008a).

In addition to the „classical Kostmann“-phenotype of SCN, several disorders combining congenital neutropenia and hypopigmentation have been described and have enabled an understanding of the complex interplay between the biology of lysosomes in pigmentation and immune regulation (reviewed in (Stinchcombe et al., 2004)). Four distinct albinism-neutropenia disorders have been described to date: Chédiak-Higashi syndrome (CHS), Griscelli syndrome type 2, Hermansky-Pudlak syndrome type 2 (HPS2) and p14 deficiency.
In contrast to SCN, mature neutrophils are found in bone marrow smears from these patients, while a paucity of neutrophils is present in peripheral blood. Chédiak-Higashi syndrome (Chediak, 1952; Higashi, 1954) is characterized by hypopigmentation, bleeding diathesis and a complex immunodeficiency caused by defective NK cell function and neutropenia (Grenda and Link, 2006). On a molecular level, CHS is caused by mutations in lysosomal trafficking regulator (LYST/CHS) gene (Barbosa et al., 1996); however, some patients with a CHS-like clinical phenotype do not show mutations in LYST/CHS, suggesting that there may be more genetic defects leading to a CHS phenotype. HPS2 is a rare disorder characterized by oculocutaneous albinism, defective thrombocyte granules, and bleeding diathesis in association with congenital neutropenia. The molecular defects are mutations in the AP3B1 gene, encoding a protein of the heterotrimeric adaptor protein 3 (AP3) complex which is crucial for the control of intracellular, vesicular cargo transport (Dell’Angelica et al., 1999). Finally, p14 deficiency has been described very recently as a syndrome of short stature, hypogammaglobulinemia, reduced numbers of class-switched B lymphocytes and defective cytotoxic T cell function, caused by mutations in the p14 (MAPBPIP) gene. P14 is required for proper assembly of late endosomes, as p14 deficient cells show an abnormally scattered distribution of late endosomes and consecutively altered mitogen-activated protein kinase signal transduction (Bohn et al., 2006; Teis et al., 2006).

### Molecular pathophysiology of severe congenital neutropenia

The molecular pathophysiology underlying different genetic subforms of SCN has remained largely unclear. The most striking phenotype of SCN is the observed, so-called „maturation arrest“ of myeloid cells in the bone marrow. Initially, this maturation arrest was thought to be the result of defective cytokine signaling. However, the serum of SCN patients contains normals or increased levels of G-CSF and G-CSF receptors on neutrophils, with no impaired biological activity of G-CSF (Kyas et al., 1992). More recently, the concept that an intrinsic defect of granulocyte differentiation underlies the neutropenia phenotype has gained support, and Skokowa et al. were able to demonstrate that myeloid progenitor cells from SCN patients have markedly decreased levels of lymphoid enhancer factor-1 (LEF-1) (Skokowa et al., 2006), a transcription factor with multiple target genes including C/EBPα. LEF-1 has a recognized, critical role in myelopoiesis (Mueller and Pabst, 2006), and ectopic expression of LEF-1 was able to overcome the observed maturation arrest in SCN patient cells in vitro (Skokowa et al., 2006). An alternative hypothesis suggests that the observed maturation „arrest“ may rather reflect increased premature cell death at the level of promyelocyte stage of differentiation (Carlsson et al., 2006). Intriguingly, several studies have shown that neutrophils or bone marrow precursor cells from SCN patients show a phenotype of enhanced apoptosis as compared to cells from healthy individuals, independent of the underlying genetic etiology (Boztug et al., 2009; Carlsson et al., 2004; Grenda et al., 2007; Klein et al., 2007).

A recent concept is that conditions which lead to disturbance of the endoplasmic reticulum homeostasis such as accumulation of misfolded proteins can induce activation of an intracellular signaling cascade called „unfolded protein response“, which may ultimately result in apoptosis. This mechanism needs to be tightly controlled so that cells can ensure the quality and abundance of potentially dangerous proteins. To prevent misfolding and overabundance of mutated molecules or in response to alterations in the cellular status, sensors facing the ER lumen and effectors which convey the signal to other compartments of the cell. Three canonical pathways of ER stress transducers have been identified, namely inositol-requiring protein 1a (IRE1α), activating transcription factor-6 (ATF6), and protein kinase RNA (PKR)-like ER kinase (PERK), respectively (reviewed in (Ron and Walter, 2007)). There is increasing evidence that ER stress plays a critical pathophysiological role in a variety of diseases including diabetes mellitus, cancer or neurodegenerative disorders (reviewed in (Lin et al., 2008; Todd et al., 2008)). In case of ELA2 deficient SCN, activation of the UPR has been documented in by independent groups of investigators (Grenda et al., 2007; Köllner et al., 2006). However, it is at present unclear whether this concept applies to other genetic forms of SCN as well. Of note, as part of our very recent discovery of human G6PC3 deficiency as a novel SCN syndrome, we could document the importance of an adequate control of glucose homeostasis for the endoplasmic reticulum in neutrophils (Boztug et al., 2008a).
Taken together, the molecular mechanisms of neutrophil apoptosis in various genetic subforms are thus far incompletely understood. Nonetheless, recent studies have provided evidence of activation of the unfolded protein response in SCN, and it is an interesting concept that this may provide a unifying explanation of the neutropenia seen in SCN of different genetic etiology. Our findings may also help to understand general pathophysiologic pathways which may be applicable to other pathologies such as neurodegenerative diseases or cancer.

References


