

Homeostatic Regulation of Blood Neutrophil Counts

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Blood neutrophil counts are determined by the differentiation and proliferation of precursor cells, the release of mature neutrophils from the bone marrow, margination, trafficking and transmigration through the endothelial lining, neutrophil apoptosis, and uptake by phagocytes. This brief review summarizes the regulation of blood neutrophil counts, which is in part controlled by G-CSF, IL-17, and IL-23. Neutrophils are retained in the bone marrow through interaction of CXCL12 with its receptor CXCR4. The relevance of this mechanism is illustrated by rare diseases in which disrupting the desensitization of CXCR4 results in failure to release mature neutrophils from bone marrow. Although blood neutrophil numbers in inbred mouse strains and individual human subjects are tightly controlled, their large variation among outbred populations suggests genetic factors. One example is benign ethnic neutropenia, which is found in some African Americans. Reduced and elevated neutrophil counts, even within the normal range, are associated with excess all-cause mortality. *The Journal of Immunology*, 2008, 181: 5183–5188.

Neutrophilic granulocytes (neutrophils), the most abundant but also very short-lived human white blood cells, act as first defenders against infections (1). Neutrophil turnover is rapid, $\sim 10^9$ cells per kilogram of body weight leave the bone marrow per day in healthy humans (2, 3). In these studies, bone marrow postmitotic transit time as determined by maximal blood neutrophil radioactivity after a pulse of [^3H]thymidine was found to be 7 days (2, 3). The transit time in rabbits and mice was somewhat shorter; the peak of cell mobilization into peripheral blood occurred ~ 95 h after leaving the mitotic pool, where progenitors remained ~ 50 h (4, 5). Within the circulation, the half-life of infused, radiolabeled neutrophils was 7–10 h in humans (3, 6) and 11.4 h in mice (4). In rabbits, a shorter half-life of 3.2 h was reported (7, 8).

Neutrophil progenitor proliferation and differentiation

Neutrophils are terminally differentiated cells. Differentiation from myeloblastic and myelocytic progenitors involves tightly

regulated sequential gene expression that leads to the formation of a granule with specific protein contents (9). Hematopoietic cytokines promote neutrophil progenitor proliferation and differentiation, acting in a complex network (10). The major cytokine for neutrophil proliferation and survival is G-CSF. Mice and humans deficient in either G-CSF or its receptor suffer from profound neutropenia (11–13). G-CSF currently is the major therapeutic agent for neutropenia of iatrogenic as well as genetic and various other origins (14–16). Extensive preclinical and clinical data exist on the role of other granulopoietic cytokines such as M-CSF, GM-CSF, IL-6, IL-3, IL-17, and, most recently, IL-22 (11, 17–23), which have been reviewed elsewhere in detail (24). Genetic modification of intracellular messengers downstream of G-CSF (25) showed for example that both STAT3 and SOCS3 (suppressor of cytokine signaling 3) deficiency resulted in neutrophilia and an increased pool of late stage progenitors in the bone marrow, thus implicating an inhibitory role (26–30). The role of transcription factors and microRNA in neutrophilic differentiation has recently been reviewed (31, 32).

A number of monogenic defects associated with rare forms of congenital neutropenia in humans are known. Maturation arrest and increased cell death of neutrophil progenitor proliferation have been observed in humans with elastase gene mutations, but also in genes encoding transcription factors such as GFI-1 (growth factor independent 1), HAX1 (hematopoietic cell-specific Lyn substrate 1-associated protein X-1), and LEF-1 (lymphoid enhancer factor-1) (33).

Neutrophil mobilization from the bone marrow

Release mechanisms of hematopoietic stem cells, myeloid progenitors, and granulocytes from the bone marrow have been studied extensively under normal and emergency conditions (34–36). The interaction of SDF1 (stromal derived factor-1; CXCL12) with the chemokine receptor CXCR4 is important for neutrophil retention in the bone marrow. CXCR4 deficiency results in decreased bone marrow but increased peripheral neutrophils as identified by the marker Gr-1 (37). Physiologically, CXCR4 and CXCL12 are down-regulated by G-CSF (38, 39), but neutrophil mobilization can also be induced by anti-CXCR4 Abs and a number of peptide antagonists (40, 41). Conversely, activating mutations of CXCR4 in humans cause

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neutrophil accumulation in the bone marrow together with peripheral neutropenia, which results in a complex phenotype (WHIM syndrome: warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis) (42–44). This year, specific patients' mutations have given further insights into downstream signaling; in one WHIM patient, decreased expression of GRK3 (G protein-coupled receptor kinase 3) was observed. G protein-coupled receptor kinases are essential for desensitization of CXCR4 and subsequent neutrophil release from the bone marrow (45). Another mutation inhibited the internalization of phosphorylated CXCR4 (46). There was no evidence for altered neutrophil mobilization in selectin-deficient or β_2 integrin-deficient mice as assessed by bone marrow cellularity and the relative cell number of wild-type and *Itgb2*^{-/-} cells in mixed bone marrow chimeras (47, 48). However, in an ex vivo model of rat femoral bone perfusion, although Abs blocking selectins did not alter neutrophil mobilization, Abs to β_1 and β_2 integrins did increase mobilization in response to MIP-2 (49). Neutrophil serine protease expression correlates with neutrophil release from the bone marrow. Cathepsin G and neutrophil elastase, but also matrix metalloproteinase 9, were increased by G-CSF treatment, and inhibition by α -1-antitrypsin inhibited neutrophil release from the bone marrow (50–52). However, neither deficiencies in both cathepsin G and neutrophil elastase nor a mouse model lacking the serine proteinase activator dipeptidyl peptidase I showed altered neutrophil mobilization, thus challenging the role of serine proteases in neutrophil liberation (39).

Marginating pool

In mice, the circulating pool of neutrophils amounts to only 1–2% of the morphologically mature neutrophils in the bone marrow (53). Neutrophil homing studies have mainly depended on extracorporally labeled cells. Such data must be interpreted with caution, because partial cell activation may occur during isolation and may alter homing properties (54). In one study, approximately one-third of reinfused neutrophils were found in liver and bone marrow and ~15% in the spleen. Interestingly, the target organ depended on the collection method. Neutrophils from thioglycollate-induced peritonitis preferentially homed to the liver and bone marrow-derived neutrophils to the bone marrow when assessed after 4 h (55). Endotoxin- or cobra venom factor-mobilized neutrophils infused into rats were found in the spleen (21%), liver (22%), and lungs (14%) after 4.5 h (56). The vasculature of the lung harbors a considerable neutrophil pool. In rabbits, ~20% of ⁵¹Cr-labeled neutrophils stayed in the healthy lung and, of those, ~90% in capillaries (57). Catecholamines can mobilize marginated neutrophils. Interestingly, altered mobilization of marginated neutrophils may be a factor in ethnic neutropenia in humans; in addition to low baseline counts, affected subjects mobilized fewer neutrophils during marathon running or other strenuous exercise (58).

Adhesion and migration into tissues

Integrins and selectins are essential for initiating neutrophil exit from the blood pool (59, 60). Specific adhesion molecule deficiencies increase circulating neutrophil numbers. Mice deficient in leukocyte function-associated Ag (CD11a; *Itgal*^{-/-}) or the common chain of all β_2 integrins (CD18, *Itgb2*^{-/-}) show marked leukocytosis (61, 62). Neutrophil migration to various

tissues was reduced in *Itgb2*^{-/-}-deficient mice (62, 63). *Itgb2* silencing by neutrophil-specific microRNA recently confirmed this phenotype (64). Mild neutrophilia was also found in mice deficient for P-selectin (*Selp*^{-/-}) (65, 66), which was more severe when both E- and P-selectin (*Selp*^{-/-}*Sele*^{-/-}) or all selectins (*Selp*^{-/-}*Sele*^{-/-}*Sell*^{-/-}) were absent (48, 66). Absence of an enzyme required for selectin glycosylation, core 2 β -1,6-N-acetylglucosaminyltransferase (*Core2*^{-/-}), resulted in neutrophilia (67).

Neutrophilia in adhesion molecule-deficient mouse strains was initially thought to be caused by passive neutrophil accumulation in blood vessels. To test whether adhesion molecule-deficient neutrophils accumulated more than wild-type cells, several groups used mixed *Itgb2*^{-/-} and wild-type bone marrow transplants into wild-type mice. Surprisingly, the percentages of wild-type and *Itgb2*^{-/-} neutrophils in peripheral blood and in bone marrow were very similar (47, 68, 69). Even a small proportion of wild-type cells was sufficient to normalize blood neutrophil levels. Proliferation measured by BrdU incorporation of Gr1-positive bone marrow cells did not differ between wild-type and *Itgb2*^{-/-} cells 6 mo after transplantation. This argues against intravascular accumulation or autonomous proliferation as reasons for neutrophilia in *Itgb2*^{-/-} mice.

In humans, leukocyte adhesion deficiencies (LAD), rare diseases caused by deficiency or signaling dysfunction of β_2 integrins (LAD I), selectin ligands (LAD II), or signaling intermediates (LAD III), lead to defective neutrophil adhesion and replicate the neutrophilic phenotype of the respective gene-deficient mice (70–72).

Apoptosis, clearance, and feedback

Neutrophils are short-lived, terminally differentiated cells with a high rate of spontaneous apoptosis. Cell death is altered in the presence of inflammatory stimuli that induce the formation of reactive oxygen species, degranulation, and, under specific conditions, exocytosis of DNA (73–75). When apoptosis was induced in vivo, neutrophils were mainly found in the liver where they were phagocytosed by Kupffer cells (76). Apoptotic neutrophil phagocytosis has an anti-inflammatory role (77). Some phagocytes produce the proinflammatory cytokine IL-23, which consists of a p40 and a specific p19 subunit. IL-23 is induced in macrophages and dendritic cells by transcription factors like NF- κ B, which can be down-regulated by neutrophil phagocytosis (78, 79). Transgenic overexpression of the IL-23-specific subunit p19 in mice induced neutrophilia (80). Conversely, IL-23 deficiency or blockade with an Ab decreased neutrophil counts in normal and neutrophilic mice (81).

IL-23 is a potent inducer of IL-17, the most prominent member of a cytokine family defining the Th17-CD4 subpopulation (82). In all strains of severely neutrophilic adhesion molecule-deficient mice, elevated IL-17 levels were found and IL-17 blockade by a soluble IL-17 receptor demonstrated that their neutrophilia was indeed caused by IL-17 (47). Mice deficient in the IL-17 receptor (*Il17ra*^{-/-}) show decreased neutrophil counts (20, 83). IL-17 stimulates G-CSF secretion (84), and G-CSF levels were elevated in all neutrophilic mouse strains where the blockade of G-CSF normalized neutrophil counts (47). Closing this feedback loop, IL-23 expression in peripheral tissues was reduced by phagocytosis of apoptotic neutrophils (63). These data suggest a model where granulopoiesis is driven

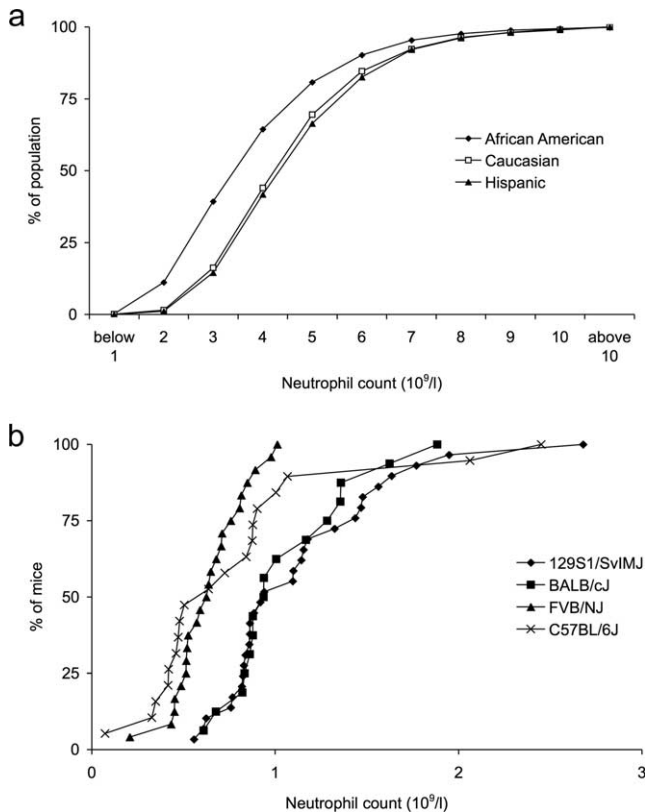


FIGURE 1. Normal range of neutrophil counts in humans and mice. *a*, Neutrophil counts from 25,000 US Americans (86), modified to show cumulative incidence. Mean counts in African Americans were significantly lower than in Caucasian or Hispanic individuals. *b*, Neutrophil counts in inbred mouse strains. Neutrophil counts calculated from white blood counts and relative neutrophil counts from 129S1/SvIMJ ($n = 29$), BALB/cJ ($n = 16$), FVB/NJ ($n = 24$), and C57BL/6J ($n = 19$) from The Jackson Laboratory (Ref. 89 and The Jackson Laboratory Mouse Phenome Database at www.jax.org/phenome).

by a cytokine cascade starting with macrophage and dendritic cell IL-23 secretion. The resulting T cell IL-17 secretion increases G-CSF levels. When neutrophils arrive in peripheral tissues their phagocytosis down-regulates macrophage IL-23 secretion and, via decreased IL-17 and G-CSF, curbs granulopoiesis (85).

“Normal” neutrophil count in humans and mice

Baseline neutrophil counts are relatively stable in individuals but have a considerable normal range in healthy humans. A survey of more than 25,000 Americans found a mean neutrophil count of $4.3 \times 10^9/l$ in adult males and $4.5 \times 10^9/l$ in females for Caucasian participants (86). In addition to environmental factors, whose influence was highlighted by a recent study showing a global decrease of neutrophil counts in an US-American population from 1958 to 2002 (87), the genetic background is important. Mean neutrophil counts are lower in African Americans: in one study, $3.5 \times 10^9/l$ in males and $3.8 \times 10^9/l$ in females (Fig. 1*a*) (86). “Benign ethnic neutropenia” is a condition found in up to 5% of African Americans and is defined as a neutrophil count $<1.5 \times 10^9/l$ without overt cause or complication (86, 88). Little is known about the genetic factors that influence this difference or human steady state granulopoiesis within the normal range.

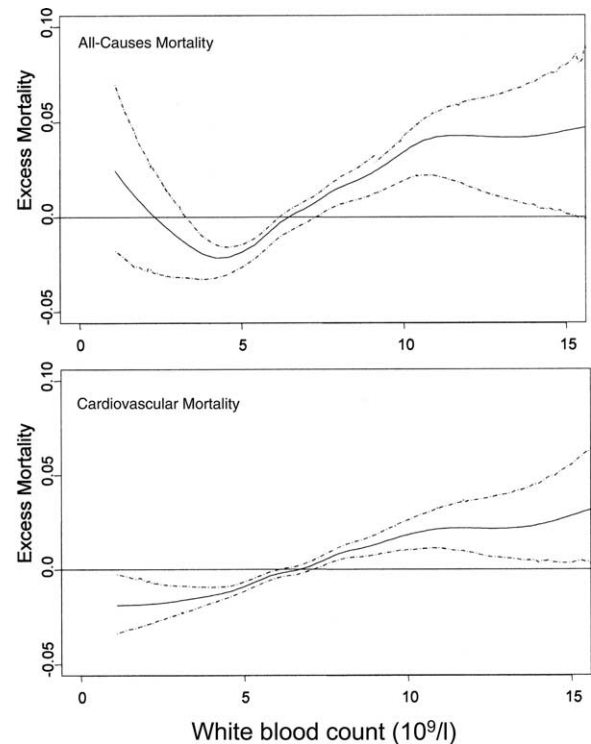


FIGURE 2. Relationship between excess mortality and WBC. Nearly 4000 individuals from the Baltimore/Washington area were observed from 1958–2002. Excess mortality as the difference between observed and expected mortality hazard over time is plotted against WBC. The dashed lines represent the 95% confidence intervals (with permission from the authors of Ref. 87).

Variation was also seen between different inbred mouse strains. Neutrophil counts from four commonly used mouse strains are given in Fig. 1*b* (Ref. 89 and The Jackson Laboratory Mouse Phenome Database at www.jax.org/phenome). Whole genome association studies of F_2 intercrosses in mice and swine revealed chromosomal regions associated with blood neutrophil counts (90–92), some of them harboring coding regions for cytokines such as IL-2, IL-15, IL-12, and chemokines such as CXCL8. However, specific mutations leading to functional alterations of these cytokines remain to be determined.

Clinical relevance of baseline neutrophil counts

Neutrophilia is a classical indicator of acute inflammation of infectious or multiple other causes such as acute arteriosclerotic events or trauma, whereas idiopathic and acquired (e.g., drug-induced) forms of neutropenia predispose to infections (14, 93). However, total white blood cell counts (WBCs),³ which are mainly determined by neutrophil counts in healthy humans, are also relevant in the absence of acute events. Increased WBCs have long been associated with increased all-cause mortality (94–98). A prospective study conducted over 44 years revealed a J-shaped association curve of neutrophil, but not lymphocyte, count and all-cause mortality (87) (Fig. 2).

Neutrophils are the first defense against invading microorganisms. Increased susceptibility to common pathogens has usually been attributed to extremely low counts ($<0.5 \times$

³ Abbreviation used in this paper: WBC, white blood cell count.

$10^9/l$) (14), and individuals with “low normal” counts or ethnic neutropenia have not been reported to be at increased risk as long as counts are not further decreased. However, the probability of contracting tuberculosis from patients with open pulmonary disease was inversely correlated with baseline neutrophil counts (94). In contrast, an increased total WBC and neutrophil count has been shown to be an independent risk factor for cardiovascular mortality in a number of studies and subsequent metaanalyses (87, 96, 99–103). Few data exist on neutrophil counts and cancer mortality, but the National Health and Nutrition Examination Survey study (National Center for Health Statistics, Hyattsville, MD) shows a higher total WBC as an independent risk factor for total cancer mortality (104). However, it remains unclear whether the elevated numbers of circulating neutrophils are causative of the observed increase in mortality or rather a measure of ongoing subclinical inflammation (105).

Summary

Stable neutrophil blood counts are the result of a highly dynamic feedback system. The study of genetically altered mice and monogenic diseases in humans has given insight into some of the mechanisms involved. However, neutrophil counts in healthy humans are regulated by a variety of environmental and genetic factors, most of which remain currently unknown. As elevated counts within the normal range are associated with excess mortality, elucidation of factors involved in steady-state neutrophil regulation might have clinical relevance.

Disclosures

The authors have no financial conflict of interest.

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