

White blood cells 1: non-malignant disorders

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- Disorders of white cells are very common in clinical practice.
- White-cell development and numbers are controlled by a mixture of external stimuli including cytokines, matrix proteins, and accessory cells.
- Several different white-cell lineages are recognised; each has a role in host defence.
- Both white-cell deficiency and overproduction can lead to disease.
- Some forms of inherited white-cell deficiency are potentially treatable with gene therapy.

The practising physician frequently sees clinical conditions that are accompanied by, or are a consequence of, disorders involving changes in the number of white blood cells. During the past two decades, a greater understanding of the cellular and molecular basis of normal and neoplastic myelopoiesis has provided new insight into the pathobiology, diagnosis, and management of these disorders.

White blood cells are an important component of the host defence system, responsible for protection against bacteria, fungi, viruses, and invading parasites. An intricate cytokine network and hierarchy of progenitor cells maintain baseline myelopoiesis and also allow rapid adjustment in the rates of production of these cell types that occur in response to acute and chronic stress.^{1,2}

Normal physiology

Production of white blood cells

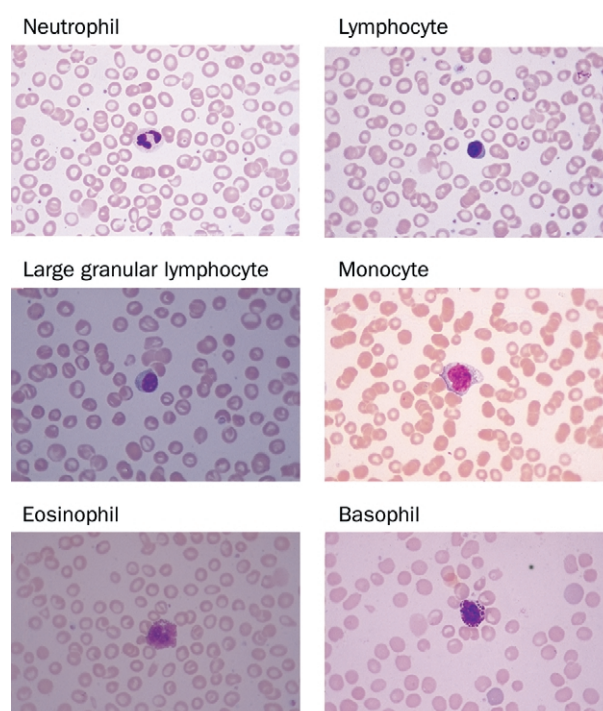
White blood cells originate from pluripotent haemopoietic stem cells.^{1,2} Under the influence of various external stimuli (cytokines, matrix proteins, and accessory cells), stem cells develop into haemopoietic progenitor cells of various lineages.² Growth factors that regulate the development of particular populations of white blood cells have been identified.^{1,2}

For neutrophils, production involves several different growth factors, including granulocyte-colony-stimulating factor (G-CSF), granulocyte-macrophage-colony-stimulating factor (GM-CSF), interleukin 3, and macrophage-colony-stimulating factor (M-CSF). In steady-state granulocyte production, G-CSF rather than GM-CSF is the most important lineage-specific factor according to findings in knockout mice. Similarly, for monocytes, both GM-CSF and M-CSF control number and function in vitro, but in mice only M-CSF deficiency leads to profound monocytopenia and macrophage deficiency. The production of eosinophils depends on GM-CSF and interleukins 3 and 5. Interleukin 5, however, is the most lineage-specific factor and acts by promoting eosinophil production and prolonging survival

of eosinophils. Although many growth factors affect B-cell lymphopoiesis, studies of mice without the genes for interleukin 7 or its receptor showed that only interleukin 7 is an obligate B-cell growth factor. However, fetal liver kinase 2 (FLK-2) ligand also seems to be important in B-cell production, since knockout mice have a 50% reduction in pro-B cells and 25% reduction in the pre-B-cell compartment. The control of T lymphocytes is a complex process; although interleukins 2 and 15 both have important roles in T-cell growth and activation, interleukin-2-knockout mice do not have lymphopenia.

Characteristics of lineages (figure)

Neutrophils make up more than half of all leucocytes and are the dominant class of circulating phagocytes (normal count $1.8-7.7 \times 10^9/L$).³ They are the front line in defence against bacterial infections. The largest pool of neutrophils is in the marrow (reserve pool), and a small number circulate in the peripheral blood (circulating pool); a number similar to that of the circulating pool exists in tissues (tissue pool). The circulating pool can be



Examples of each type of white cell

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further subdivided into two roughly equal compartments: a margined pool of cells loosely adherent to vascular endothelium, and a freely circulating pool.⁴ Only the latter can be counted. Stress (exercise) causes a transient increase in the freely circulating pool.⁴ Corticosteroids promote release of neutrophils from the marrow storage pool into the circulation and also inhibit movement of these cells from the blood into the tissues, resulting in an increased measured white-cell count. Intravascular activation of neutrophils (C5a, endotoxin) results in increased margination and hence a decrease in the measured neutrophil count. The half-life of a circulating neutrophil is short (6–8 h).⁵

Lymphocytes represent about a third of the white blood cells in the peripheral blood ($1.0\text{--}4.0\times 10^9/\text{L}$). Most have a compact rounded or gently notched nucleus with scant agranular cytoplasm. Two thirds are T cells, which participate in cell-mediated immune responses. The remainder are B cells, which are programmed to produce antibodies.

B and T lymphocytes cannot be distinguished morphologically. Some 10% of lymphocytes are large granular lymphocytes, characterised by abundant cytoplasm and reddish granules. These cells are called natural killer cells because of their ability to destroy virus-infected and HLA-incompatible target cells.

3–8% of circulating leucocytes are monocytes ($0.3\times 10^9/\text{L}$), which are characterised by their large size and folded nuclei. After migration into extravascular tissues, they increase in size and acquire the morphological characteristics of tissue macrophages. Macrophages and monocytes play an important part in regulating the afferent and efferent components of the immune system.⁶

Mature eosinophils, which make up 5–10% of granulocytes ($0.2\times 10^9/\text{L}$), have a bilobed nucleus and numerous orange cytoplasmic granules. Eosinophils have substantial proinflammatory and cytotoxic activity and play an important part in the pathogenesis of various allergic, parasitic, and neoplastic disease processes.⁴

Basophils are the least common granulocytes and are distinguished from eosinophils by large metachromatic (purple-black) granules rich in histamine, serotonin, and leukotrienes.⁷ Mast cells are related to, but distinct from, basophils. Basophils are bilobed, whereas mast cells are long-lived cells that reside in tissues rather than peripheral blood and are capable of cell division. Both cell types are involved in immediate and cutaneous hypersensitivity reactions including asthma, urticaria, allergic rhinitis, and anaphylaxis.

Leucocytosis

Definition

Leucocytosis is used to mean an increase in the white-blood-cell count to more than 2 SDs above the mean of one or more subsets of circulating white blood cells. It may reflect a primary disorder of bone-marrow production—either congenital or an acquired malignant disorder—or a secondary response to a disease process, drug, or toxin. Malignant disorders are discussed in the second paper on white cells in this series.

Congenital forms of neutrophilia (panel 1)

Leucocyte adhesion deficiency is a rare congenital disorder of neutrophil function involving a defect in expression of

specific integrins of leucocytes of affected individuals.⁸ As a result, phagocytes do not migrate from the bloodstream to sites of infection. The disorder presents as a persistent granulocytosis with neutrophil counts of $12\text{--}100\times 10^9/\text{L}$ ($12\,000\text{--}100\,000/\mu\text{L}$), recurrent cutaneous abscesses, and periodontal infections or gingivitis.⁸ Since neutrophils are unable to migrate to tissues, abscesses and other sites of infection are devoid of pus despite the striking neutrophilia. Granulocytes show very low expression (or absence) of CD11b and CD18; this feature establishes the diagnosis. Therapy includes prophylactic antibiotics and aggressive treatment of periodontal disease. In patients with severe leucocyte adhesion deficiency, stem-cell transplantation is recommended.⁹

Chronic idiopathic neutrophilia is a chronic form of leucocytosis that occurs in people who are otherwise healthy. Individuals with leucocyte counts of $11\text{--}40\times 10^9/\text{L}$ have been followed up for as long as 20 years without development of any associated clinical disease.⁹

Hereditary neutrophilia, a syndrome of high leucocyte counts ($20\text{--}70\times 10^9/\text{L}$), splenomegaly, and widened diploë of the skull has been described in a few families.¹⁰ Patients have high leucocyte alkaline phosphatase activities and no propensity to infection. The disorder seems to show autosomal dominant inheritance.

Leukaemoid reactions occur in congenital syndromes. This term is used to describe the presence of leucocytosis ($>50\times 10^9/\text{L}$) characterised by an increase in early neutrophil precursors in the blood with a pronounced “left shift” and the presence of myelocyte, metamyelocyte, and band forms. In severe reactions, even more immature cells, promyelocytes and myeloblasts, may circulate in the blood. In contrast to acute leukaemia, there is an orderly maturation and proliferation of all normal myeloid elements in the bone marrow. Leukaemoid reactions can be distinguished from chronic myeloid leukaemia by a normal leucocyte alkaline phosphatase activity and absence of the Philadelphia chromosome. Some infants with Down’s syndrome have transient leukaemoid reactions that may be difficult to differentiate initially from acute leukaemia. These children also have an exaggerated leukaemoid response to stress.

Familial cold urticaria and leucocytosis is an unusual syndrome of leucocytosis, fever, urticaria, rash, and muscle and skin tenderness on exposure to cold. It seems to have a dominant pattern of inheritance.¹¹

Secondary neutrophilia

Acute infection—A slight rise in leucocyte number with a left shift is seen commonly in association with many acute bacterial infections. It may also be associated with severe viral diseases. Morphological changes in the neutrophil occur during infection, including the development of toxic granulation, Döhle bodies, and cytoplasmic vacuoles.

Panel 1: Non-malignant causes of neutrophilia

Congenital forms	Secondary causes
Leucocyte adhesion deficiency	Acute infection
Chronic idiopathic neutrophilia	Chronic inflammation
Hereditary neutrophilia	Leukaemoid reaction
Leukaemoid reaction in congenital syndromes	Stress neutrophilia
Familial cold urticaria	Drug-induced neutrophilia
	Marrow stimulation
	Asplenia, hyposplenism

Chronic inflammatory processes result in stimulation of bone-marrow granulocyte production. In these disorders the degree of neutrophilia is moderate, and it may be associated with monocytosis.

Leukamoid reactions causing neutrophilia also occur in patients with osteomyelitis, empyema, septicaemia, and tuberculosis. Non-infectious causes include carcinoma (lung, stomach, breast), Hodgkin's disease, juvenile rheumatoid arthritis, and dermatitis herpetiformis. Metastatic infiltration of the bone marrow may also cause the presence of nucleated red blood cells.

Stress neutrophilia can occur within minutes of exercise or emotional or physical stress, or after surgery, seizures, or epinephrine injection. The increase in neutrophil count, small in most cases, is thought to be related to movement of neutrophils from the marginated pool into the circulation.

Drugs—Steroids stimulate the release of neutrophils from the bone marrow and result in a chronic neutrophilia. This drug-induced neutrophilia can be distinguished from neutrophilia due to an acute infection by the lack of increased proportion of band forms. β agonists, such as theophylline, produce an acute neutrophilia by releasing neutrophils from the marginated pool. Lithium and tetracycline also cause leucocytosis.¹² The clinical use of G-CSF and GM-CSF has become more widespread, and these cytokines can result in clinically significant neutrophilia if administered in high dose; therefore, blood counts must be carefully monitored during growth-factor administration. Typically, growth-factor use results in a left shift in the complete blood count (ie, a predominance in the blood of immature forms of neutrophils). The haemopoietic growth factors are used clinically in various ways: after chemotherapy, to prevent episodes of neutropenic fever and to allow for the delivery of chemotherapy in a timely way; after autologous and allogeneic bone-marrow transplantation, to limit the time to engraftment and recovery from neutropenia; in bone-marrow failure including myelodysplastic syndromes; in patients with primary or secondary neutropenia resulting from the disorders discussed below; and in patients with AIDS or HIV infection.

Marrow stimulation—Haemolytic anaemias or immune thrombocytopenia result in chronic bone-marrow stimulation with resultant leucocytosis. Patients with sickle-cell anaemia commonly have leucocyte counts in the range $12\text{--}15 \times 10^9/\text{L}$; consequently, these patients often have an exaggerated increase in white-blood-cell counts during infections.

Asplenia—Functional or pathological asplenia, after surgical removal of the spleen, or in various congenital and acquired diseases, may result in moderate neutrophilia.

Lymphocytosis

The average absolute lymphocyte count during the first year of life ranges from 5.0 to $7.0 \times 10^9/\text{L}$. The count declines gradually to adult values ($2.0 \times 10^9/\text{L}$). Lymphocytosis is defined as a count above $9.0 \times 10^9/\text{L}$ in infants and above $4.0 \times 10^9/\text{L}$ in adults. Malignant causes of lymphocytosis are discussed in the next paper in this series.

Lymphocytosis occurs commonly after many viral infections (panel 2). In infectious mononucleosis caused by Epstein-Barr virus, there is lymphocytosis with characteristic large atypical lymphocytes in the blood

Panel 2: Secondary causes of lymphocytosis

Viral infections: infectious mononucleosis; cytomegalovirus; hepatitis; mumps; varicella; rubeola; rubella; herpes simplex virus; herpes zoster; influenza; roseola; acute infectious lymphocytosis
Bacterial infections: pertussis, rickettsial infections; tuberculosis; syphilis; brucellosis
Drug-induced
Serum sickness

because, although the virus infects B lymphocytes, the atypical lymphocytes are primarily CD8 T lymphocytes and natural killer cells. The clinical presentation of Epstein-Barr virus infection in young adults may be confused with acute lymphoblastic leukaemia; the two disorders are distinguished on the basis of bone-marrow examination, lymphocyte immunophenotyping, and serological findings.¹³ The diagnosis can be made by a rapid slide test for heterophil antibodies (eg, Monospot). If this test is negative, but the clinical suspicion of infectious mononucleosis is high, the serum sample should be tested for antibodies specific for Epstein-Barr virus.

Lymphocytosis is rarely seen in bacterial infections, with the exception of *Bordetella pertussis* infection, in which the lymphocyte count typically rises to more than $10.0 \times 10^9/\text{L}$. Drug-induced immunological reactions may result in lymphocytosis with the appearance of many atypical lymphocytes that are large, irregular, and deeply basophilic in many cases. A similar picture can occur in serum sickness after administration of antithymocyte globulin.

Monocytosis

An absolute monocyte count greater than $0.9 \times 10^9/\text{L}$ can occur as a result of several chronic infectious or inflammatory disorders (panel 3). Several leukaemic disorders are also characterised by monocytosis and are a result of a primary stem-cell defect. Chronic myelomonocytic leukaemia is a disorder of older people, with features of both a myelodysplastic disorder and a chronic myeloproliferative disease.¹⁴ These patients present with hypercellular marrow, various degrees of dysplastic haemopoiesis, thrombocytopenia, and splenomegaly. By definition, these patients have a peripheral monocyte count over $1.0 \times 10^9/\text{L}$ and increased numbers of monocytes in the bone marrow. Very high

Panel 3: Causes of monocytosis

Primary causes

Congenital disorders
Cyclical neutropenia
Congenital agranulocytosis

Secondary causes

Recovery phase from neutropenia
Chronic infections: tuberculosis; subacute bacterial endocarditis; fungal infections; brucellosis; kala-azar; trypanosomiasis
Autoimmune disorders: rheumatoid arthritis; systemic lupus erythematosus; polyarteritis; ulcerative colitis; regional enteritis; sarcoidosis
Malignant diseases: ovarian, gastric, and ovarian cancer; non-Hodgkin lymphoma; Hodgkin's disease

Malignant causes

Chronic myelomonocytic leukaemia
Acute myeloid leukaemia (M5 subtype)
Juvenile myelomonocytic leukaemia
Chronic myeloid leukaemia
Myeloproliferative disease of monosomy 7

monocyte counts can be associated with the development of pericardial, pleural, or joint effusions and ascites. Many patients with chronic myelomonocytic leukaemia have clonal cytogenetic abnormalities, and the disease can progress to acute myeloid leukaemia.

Juvenile myelomonocytic leukaemia occurs exclusively in children younger than 4 years. Children present with malaise, bleeding, and fever. On physical examination, pallor, hepatosplenomegaly, lymphadenopathy, and a facial eczematoid rash are found.¹⁵ Laboratory findings include anaemia, thrombocytopenia, a raised leucocyte count ($50\text{--}100 \times 10^9/\text{L}$) with prominent monocytosis. Juvenile myelomonocytic leukaemia occurs with increased frequency in patients with neurofibromatosis. The leukaemic cells seem to be hypersensitive to GM-CSF, tumour necrosis factor α and interleukin- 1β .¹⁵ The clinical course is rapid, with death occurring from progressive marrow failure or leukaemic transformation within 9 months. The myeloproliferative disease of monosomy 7 has a similar clinical presentation but a more indolent course with a latent period of 3–6 years before transformation to acute leukaemia.¹⁶

Eosinophilia

Eosinophilia describes an absolute eosinophil count greater than $0.5 \times 10^9/\text{L}$. Most cases are secondary to an underlying inflammatory, allergic, or atopic disorder, although eosinophilia is observed in patients with parasitic infections as well as several malignant disorders, including Hodgkin's and non-Hodgkin lymphoma. The hypereosinophilic syndrome is a group of disorders of unknown cause characterised by excessive production of eosinophils associated with organ infiltration.¹⁷ This syndrome results in end-organ damage, primarily involving the heart, leading to eosinophilic endomyocardial fibrosis and associated thromboembolic complications. The diagnostic criteria for hypereosinophilic syndrome include: persistent eosinophilia ($>1.5 \times 10^9/\text{L}$) for longer than 6 months; exclusion of other secondary causes of eosinophilia; and signs and symptoms of organ dysfunction due to eosinophilia, asthma, pulmonary infiltrates, sinusitis, neuropathy, and vasculitis.¹⁸ Patients with hypereosinophilic syndrome do not have asthma, and many have more profound eosinophilia than patients with Churg-Strauss syndrome, although distinction of the two is difficult.

Basophilia

An absolute basophil count greater than $0.2 \times 10^9/\text{L}$ may be observed with acute hypersensitivity reactions, chronic infections, and inflammatory disorders (tuberculosis, rheumatoid arthritis, ulcerative colitis) or with viral infections (influenza, varicella).⁷ Many patients with chronic myeloproliferative disorders (chronic myeloid leukaemia, polycythaemia vera) present with basophilia, which may increase in severity as the disorder progresses. Mast-cell leukaemia is a rare syndrome characterised by the presence in the peripheral blood of large numbers of mast cells of atypical appearance, associated with leucocytosis and granulocytosis. These patients generally survive for less than 6 months.

Leucopenia

One or more lineages may be affected in leucopenia, and there may be an imbalance in the white-cell

subpopulations rather than a decrease in the absolute total count.

Neutropenia

Neutropenia is defined as an absolute neutrophil count of more than 2 SDs below a normal mean value. Normal absolute neutrophil counts vary among ethnic groups; the lower limit of normal for white people is $1.5 \times 10^9/\text{L}$, whereas black people have slightly lower counts, with a lower limit of normal of about $1.2 \times 10^9/\text{L}$. The lower absolute neutrophil count in black people has been attributed to a relative decrease in the size of the marrow storage pool.

The degree of neutropenia predicts the risk of serious bacterial infections.¹⁹ Only patients with severe neutropenia (count $<0.5 \times 10^9/\text{L}$) have an increased risk of developing life-threatening infections.¹⁹ Mild (counts of $1.0\text{--}1.5 \times 10^9/\text{L}$) or moderate ($0.5\text{--}1.0 \times 10^9/\text{L}$) neutropenia may not predispose a patient to life-threatening infection, but should not be ignored since it may be the consequence of an underlying haematological disorder. The duration of the neutropenia commonly defines the risk to the patient. The acute onset of severe neutropenia is frequently associated with a high risk of infection and presents as fever and sepsis, whereas patients with severe neutropenia of more gradual onset may have far less threatening symptoms at presentation. Severe neutropenia associated with fever of recent onset is a medical emergency requiring aggressive investigation and treatment.

There are well-recognised inherited forms and acquired forms of neutropenia (panel 4). Some cases defy classification. Such patients are generally followed up for long periods before the underlying cause of their neutropenia becomes apparent. Neutropenia can, of course, be a manifestation of a more extensive marrow failure state such as aplastic anaemia, Fanconi's anaemia, myelodysplasia, or acute leukaemia.

A large number of primary inherited haematological disorders present as neutropenia, predominantly during childhood.

Congenital agranulocytosis (Kostmann's syndrome) is characterised by early onset of life-threatening bacterial infections, severe neutropenia, and maturation arrest of marrow granulopoiesis at the myelocyte/promyelocyte stage.^{20,21} This disorder generally shows autosomal recessive inheritance, but sporadic and autosomal dominant patterns have been described. Affected children develop frequent and life-threatening infections. Long-

Panel 4: Causes of neutropenia

Inherited forms	Acquired forms
Ethnic or benign familial neutropenia	Drug or toxin induced
Kostmann's syndrome (severe infantile agranulocytosis)	Postinfectious
Cyclical neutropenia	Autoimmune diseases
Schwachman-Diamond-Oski syndrome	Leukaemic disorders
Chediak-Higashi syndrome	T γ lymphoproliferative disorder
Reticular dysgenesis	Hypersplenism
Dyskeratosis congenita	Nutritional deficiency
Hyperimmunoglobulin-M syndrome	Pure white-cell aplasia
	Paroxysmal nocturnal haemoglobinuria
	Metabolic disorders
	Isoimmune neonatal neutropenia
	Autoimmune neutropenia

term G-CSF therapy is effective and well tolerated, and leads to a sustained neutrophil response (associated with a reduction in the rate of severe infections and the use of intravenous antibiotics) in 91% of cases.²¹ During long-term follow-up, 16 of 220 patients with congenital agranulocytosis maintained on chronic G-CSF therapy developed acute myeloid leukaemia or a myelodysplastic disorder.^{21,22} This transformation is thought to be a consequence of the intrinsic stem-cell defect that leads to the congenital agranulocytosis. Mutations of the receptor for G-CSF resulting in the truncation of the C-terminal maturation domain are associated with progression from congenital agranulocytosis to myelodysplasia or acute myeloid leukaemia.²³ For patients who do not respond to G-CSF, allogeneic stem-cell transplantation is the only curative approach.

Cyclical neutropenia is a rare disorder caused by a stem-cell regulatory defect; it is characterised by a transient severe neutropenia that occurs roughly every 21 days.²⁴ The nadir neutrophil count lasts 3–7 days and is frequently associated with monocytosis. The nadir absolute neutrophil count is generally between zero and $0.2 \times 10^9/L$. The marrow is characterised by transient arrest at the promyelocyte stage before each cycle. Cycling of platelets and red-cell production is also observed in some cases. The patients present with a history of recurrent fever, pharyngitis, stomatitis, and, in some cases, life-threatening bacterial infections. Most patients present in childhood but there is also an adult-onset form of the disease.²⁰ The familial form seems to be inherited in an autosomal dominant pattern. Genetic linkage analysis of affected families has allowed the locus for cyclical neutropenia to be mapped to chromosome 19p13.3, where several different mis-sense and splicing mutations have been found in the gene encoding neutrophil elastase, a chymotryptic serine protease of neutrophils and monocyte granules.²⁵ G-CSF therapy of cyclical neutropenia is not associated with the development of acute myeloid leukaemia or myelodysplasia.^{21,26}

Schwachman-Diamond-Oski syndrome is an autosomal recessive syndrome characterised by the triad of neutropenia, metaphyseal dysplasia, and pancreatic insufficiency.²⁰ The absolute neutrophil count is less than $0.5 \times 10^9/L$ in most patients, and many are thrombocytopenic and anaemic. Other physical abnormalities, including short stature, cleft palate, and microcephaly, are common. Some patients eventually develop acute leukaemia or aplastic anaemia, which suggests an underlying stem-cell defect. The marrow is hypocellular at presentation in many patients. The gastrointestinal manifestations respond to pancreatic enzyme replacement and generally resolve by age 5–10 years.^{20,22} G-CSF therapy has resulted in increases to normal of the absolute neutrophil count and is the treatment of choice in patients with recurrent infections.^{21,22} Stem-cell transplantation is reserved for patients who develop aplastic anaemia or acute leukaemia.

Chediak-Higashi syndrome is a rare autosomal recessive disorder characterised by oculocutaneous albinism, progressive neurological abnormalities, and large blue-grey granules in the cytoplasm of neutrophils, eosinophils, basophils, and platelets. Patients develop severe neutropenia due to ineffective granulopoiesis and die, mostly in childhood, from infections involving the skin or pulmonary tract or an unusual lymphoproliferative disorder. Mutations in the lysosomal trafficking regulator,

or *LYST* gene, located on chromosome 1q43, have been implicated as the cause of the syndrome. Mutations in *LYST* result in defective T-cell signalling, which may lead to the development of the associated lymphoproliferative syndrome seen in patients with Chediak-Higashi disease.^{27–29}

Reticular dysgenesis is characterised by agranulocytosis, lymphoid hypoplasia, and thymic dysplasia associated with normal erythropoiesis and thrombopoiesis. Patients have low serum concentrations of IgG and IgM and die from overwhelming bacterial and viral infections.

Dyskeratosis congenita is a rare X-linked bone-marrow-failure syndrome characterised by abnormal skin pigmentation, nail dystrophy, and mucosal leucoplakia.^{30,31} More than 80% of patients develop bone-marrow failure, which is the major cause of premature death. Development of the syndrome is linked to mutations in the *DKC1* gene located at Xq28. The gene product, dyskerin, is homologous to a yeast protein involved in ribosomal RNA biosynthesis; this observation may provide an insight into the pathogenesis of aplastic anaemia.^{30,31}

Hyperimmunoglobulin-M syndrome is an X-linked disorder characterised by lymphoid hyperplasia and low concentrations of IgA and IgG but low concentrations of IgM.³² Many of these patients develop severe neutropenia. This disorder has been attributed to a genetic defect in the T-cell CD40 ligand.³³ Patients die of overwhelming infection by age 5 years unless they receive intravenous immunoglobulins and long-term G-CSF therapy.

Drugs—A growing number of drugs have been associated with the development of neutropenia.³⁴ Most are due to dose-dependent marrow suppression or immunological mechanisms. The neutropenia generally develops within 1–2 weeks of the start of drug therapy. The most common offending agents are phenothiazines, non-steroidal anti-inflammatory agents, gold salts, ibuprofen, sulphonamides, antithyroid medications, anticonvulsants, high-dose semisynthetic penicillins, vancomycin, clindamycin, ganciclovir, and H₂ blockers. If the neutropenia is mild or moderate (absolute neutrophil count $>0.5 \times 10^9/L$) and no substitute is available for the drug, continued administration with close observation is reasonable.

Viral infections—Postinfectious neutropenia commonly accompanies viral infections. Infection with HIV and the development of AIDS are associated with mild to moderate neutropenia. In children, varicella, measles, rubella, infectious mononucleosis, and influenza may result in neutropenia. Infections with hepatitis A and B viruses, parvovirus, and cytomegalovirus also result in neutropenia. Virus-induced neutropenia generally begins within a few days of the viral infection and persists for several weeks.

Bacterial infections—Overwhelming sepsis due to various bacterial infections can lead to severe neutropenia.³⁵ This degree of neutropenia is most common in neonates and in debilitated adults, and is due to exhaustion of the neutrophil marrow reserve pool. It has a poor prognosis.

Autoimmune neutropenia, an isolated neutropenia, has been reported in patients with various autoimmune disorders, including systemic lupus erythematosus, rheumatoid arthritis, autoimmune thrombocytopenic purpura, and autoimmune haemolytic anaemia.³⁵ IgG or

Type	Inheritance	Molecular defect	Phenotype
X-linked	X-linked	Mutation in γ chain of interleukin-2 receptor	T cells absent; B cells present, but impaired function; NK cells absent
JAK3 deficiency	Autosomal recessive	Mutation in <i>JAK3</i> gene; impaired signal transduction	Low T-cell numbers; defective B-cell signalling; NK cells absent
Adenosine deaminase deficiency	Autosomal recessive	Mutations in adenosine deaminase gene; impaired purine salvage pathway	Impaired function of T and B cells
Lymphocyte-receptor gene rearrangement defects	Autosomal recessive	? defects in DNA repair genes; <i>RAG1</i> and <i>RAG2</i> gene mutations	Impaired function of T and B cells
Omenn's syndrome	Autosomal recessive	Unknown; results in autoreactive T cells that produce high amounts of interleukins 4 and 5	Impaired function of T and B cells
Reticular dysgenesis	Autosomal recessive	Unknown	Absence of both B and T cells and granulocytes from bone marrow and blood

NK=natural killer.

Types of severe combined immunodeficiency

IgM antibodies may be directed against mature neutrophils or marrow precursors.

Felty's syndrome is a well-described collection of clinical abnormalities consisting of rheumatoid arthritis, splenomegaly, and severe neutropenia. The cause has been attributed to increased neutrophil margination and inhibition of granulopoiesis mediated by antibodies to neutrophils or by T cells.^{36,37} Although many patients with this disorder have no symptoms, a proportion have serious recurrent bacterial infections. Many such patients show great improvement after G-CSF therapy during an acute infectious process and require long-term therapy.

Lymphoproliferative disease of granular (T γ) lymphocytes commonly manifests as severe neutropenia with a median age at onset of 55 years.^{36,37} It results from the clonal proliferation of either CD3-positive (T) or CD3-negative (natural killer) cells.¹⁸ The natural killer cells do not have rearranged T-cell-receptor genes, whereas the CD3-positive large granular lymphocytes have clonally rearranged T-cell-receptor genes and are thought to represent in-vivo-activated cytotoxic T lymphocytes. The disease is characterised by recurrent bacterial infections that occur as a consequence of severe neutropenia. In one series, CD3-positive lymphoproliferative disease of granular lymphocytes was found in a third of patients with Felty's syndrome.³⁷ Some patients with adult-onset cyclical neutropenia also have this disorder.^{20,22} The finding of more than $2.0 \times 10^9/L$ large granular lymphocytes persisting for more than 6 months is required to confirm the diagnosis.³⁸ There is diffuse lymphocytic infiltration of the marrow with maturation arrest of the myeloid cells. The expansion of the large granular lymphocyte population can be established by use of flow cytometry. Since the CD3-positive CD16-positive cell population accounts for less than 5% of normal cells, an increase in this cell population is characteristic. For the CD3-positive form, rearrangement of T-cell-receptor genes can be detected by Southern blotting, which confirms the clonal origin of the disorder. CD3-positive lymphoproliferative disease of granular lymphocytes generally has an indolent, but not altogether benign course. Most patients need treatment for the recurrent episodes of life-threatening infection. G-CSF therapy is effective in treating acute infections, and methotrexate, prednisone, cyclophosphamide, or cyclosporin result in improvement in neutrophil counts for sustained periods.³⁸ In contrast, the CD3-negative CD56-positive (natural killer) disorders can be clinically aggressive; they tend to occur in younger patients. These patients present with fever, massive hepatosplenomegaly, and jaundice. Blood, bone marrow, and other organs are infiltrated with lymphomatous cells. This disorder typically has a rapidly progressive course and is refractory to combination chemotherapy.

Pure white-cell aplasia is an acquired disorder characterised by severe neutropenia or a total absence of neutrophils, due to an IgG or IgM antibody directed against granulocytic precursor or progenitor cells.³² Many patients with this disorder have a history of thymoma. The marrow has no myeloid precursor cells but shows normal erythroid and megakaryocyte development and a normal karyotype. If thymectomy is not effective, immunosuppressive therapy, including steroids, intravenous immunoglobulins, cyclophosphamide, or cyclosporin may be effective.

Isoimmune neonatal neutropenia, occurring in 0.5–2.0 per 1000 livebirths, develops antenatally and can be recognised at delivery or during the first days of life.³² This diagnosis should be considered in any infant with neutropenia that persists for several days. The disorder is due to the maternal production of IgG directed against antigens on the fetal neutrophils. Sensitisation to fetal antigens can occur at any time during gestation, and the IgG antibody is transferred to the fetus across the placenta.

Other causes—Neutropenia has been associated with deficiencies of vitamin B12, folate, and copper. These disorders are characterised by ineffective granulopoiesis that is corrected by appropriate supplementation. Hypersplenism can lead to neutropenia, rarely severe enough to lead to symptoms; this neutropenia is frequently accompanied by anaemia or thrombocytopenia and the marrow is hypercellular with normal myeloid development. Complement activation leading to generation of C5a can lead to acute and chronic neutropenia.³²

Lymphopenia

Severe combined immunodeficiency is a heterogeneous group of disorders characterised by profound deficiency of both T and B lymphocytes.³⁹ These disorders may show autosomal recessive or X-linked inheritance (table). The X-linked form is the most common and is characterised by the absence of mature T cells and natural killer cells in the presence of normal numbers of poorly functioning B cells. The molecular defect in this form is a mutation in the γ chain that is common to the receptors for interleukins 2, 4, 7, 9, and 15 and accounts for 50–60% of cases of severe combined immunodeficiency.⁴⁰

A deficiency of adenosine deaminase underlies 15% of cases in total, and 30–40% of cases of the autosomal recessive form.⁴¹ Adenosine deaminase deficiency leads to accumulation of the toxic metabolites of purine metabolism, deoxyadenosine triphosphate and 2'-deoxyadenosine.⁴⁰ The different forms of severe combined immunodeficiency are clinically indistinguishable, and present in early infancy with severe recurrent infections

due to opportunistic viral and fungal infections resulting in failure to thrive. The diagnosis is a medical emergency, since correction of the defect by stem-cell transplantation or enzyme replacement with adenosine deaminase treated with polyethylene glycol leads to clinical improvement or cure.⁴² Transfer of the γ common chain into CD34-positive cells of two children with severe combined immunodeficiency has been reported.⁴³ Gene therapy treatments to correct adenosine deaminase deficiency presently are being actively investigated.⁴⁴

Lymphocyte counts below $1 \times 10^9/L$ accompany many acute infections, particularly those characterised by granulocytosis. Lymphopenia is characteristic of HIV-infected individuals, and an absolute reduction in CD4-positive T lymphocytes is one of the earliest immunological consequences of HIV infection.

Agents that mimic the genetic defect in adenosine deaminase deficiency were developed for the treatment of low-grade lymphoproliferative disorders. They act by irreversibly binding to adenosine deaminase (deoxycoformycin) or by resisting deamination in the purine salvage pathway (2-chloroadenosine). These agents can lead to severe lymphopenia lasting 1–2 years.^{45,46} Fludarabine, which inhibits ribonucleotide reductase, is now a commonly used agent for the treatment of chronic lymphocytic leukaemia.⁴⁷ Its use can lead to severe T-cell immunodeficiency and lymphopenia resulting in occasional infections with *Pneumocystis carinii* or cytomegalovirus.

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