Neutrophil dysfunction

Case studies and review[1]

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The polymorphonuclear leukocyte (PMN), or neutrophil, plays a central role in the defense of the body against acute bacterial infection. PMNs also migrate to sites of tissue necrosis and actively participate in the early stages of repair. They may have tumoricidal activity and thereby play a role in the immune surveillance which is thought to be important in controlling neoplastic disease. Two cases which illustrate defects in neutrophil motility and oxidative metabolism are described. A number of intrinsic neutrophil defects are reviewed.

Index term: Neutrophils

Cleve Clin Q 53:299–306, Fall 1986

Polymorphonuclear leukocytes (PMNs) are produced in the bone marrow and actively migrate first into the circulation and finally into tissues or other body compartments. At their site of action, they engage in a complex series of physical and chemical processes, ultimately achieving bacterial sequestration and killing, as well as engulfment and digestion of cellular debris. Problems may arise at virtually any step in the pathway of normal neutrophil function,[1–5] and the clinician should be aware of this, particularly when confronted with a patient having recurrent bacterial infections.

In this paper, we review various aspects of normal neutrophil physiology, as well as a number of recognized intrinsic neutrophil defects. The following cases illustrate two different types of defects in neutrophil function.

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0009-8787/86/03/0299/08/$3.00/0

Table 1. Results of neutrophil chemotaxis assays

Neutrophil donor	Units of migration (mean ± S.D.)	
	Random (unstimulated) migration	Stimulated migration (chemotaxis)*
Patient 1	7.0 ± 1.0	35.3 ± 6.4
Patient 2	4.5 ± 1.8	32.7 ± 2.1
Normal controls (21)	17.5 ± 3.0 (12–24)†	42.6 ± 5.8 (31–54)†

* N-formyl-methionyl-phenylalanine was used as a chemoattractant.
† Normal confidence interval.

Case reports

Case 1. A 37-year-old housewife was first seen at The Cleveland Clinic Foundation on March 30, 1984, for evaluation of allergies and recurrent sinusitis for the previous 16 years. A Caldwell-Luc operation performed in 1983 had been followed by the development of bronchial asthma. Several other sinus drainage procedures were subsequently performed. Cultures of sinus drainage had repeatedly grown *Pseudomonas* and *Staphylococcus aureus*. On January 4, 1985, she was referred to the clinical immunology section for immune evaluation because of recurrent infections. Her medications at that time included prednisone (30 mg/day), Theo-Dur (700 mg/day), Brethine (2.5 mg twice daily), and entex (one, twice daily). She gave no history of recurrent infections except in the sinuses.

On examination, her temperature was 37.2 °C. The tonsils were noted to be surgically absent. A few wheezes were heard on auscultation of the chest. There was no lymphadenopathy and there was no arthritis.

Laboratory studies disclosed the following values: complete blood count with hemoglobin, 12.0 gm/dL; hematocrit, 37.0%; and white count, 4,000/mm^3 with 74% neutrophils, 4% bands, 15% lymphocytes, 6% monocytes, 1% eosinophils, and 436,000 platelets/mm^3. Immunoglobulin levels were as follows: IgM, 233 mg/dL; IgA, 282 mg/dL; and IgG, 879 mg/dL. Lymphocyte transformation studies to nonspecific mitogens revealed slight reductions of the responses to phytohemagglutinin A (49,553; lower limit of normal, 56,280) and concanavalin A (20,917; lower limit of normal, 35,109), but a normal response to pokeweed mitogen (38,795; lower limit of normal, 11,245). Antibody reactivity against tetanus was normal at 1:32 (normal, >1.8), while antibody activity against diphtheria was borderline detectable at 0.01 U/mL. Her blood was type O, Rh+; anti-A was present at a titer of 1:16 and anti-B at a titer of 1:32. Total hemolytic complement was normal at 141 CH_{50} U/mL (normal, 70–190).

Case 2. A 21-year-old man from Colombia was first seen at The Cleveland Clinic Foundation on November 12, 1984, with a six-year history of recurrent skin infections primarily on the lower extremities. These had begun with an infected mosquito bite on the left leg. Subsequently, ulcerations over both lower extremities had occurred periodically. Culture data were not available, but these lesions responded to intermittent treatment with penicillin. Aside from two episodes of blepharitis involving the left eye (the most recent being four to five months previously), no other significant history of infections was noted. He had three siblings, all of whom were healthy.

On physical examination, he appeared to be a healthy young man in no distress. Examination of the skin revealed widely distributed small pitted scars over both legs. No other skin lesions were seen and there were no active ulcers. There was no lymphadenopathy; the tonsils were surgically absent. The tympanic membranes appeared normal. Examination of the abdomen revealed a palpable spleen tip. No other abnormalities were found.

Laboratory studies disclosed the following values: complete blood count with hemoglobin, 15.2 g/dL; hematocrit, 44.3%; platelet count, 294,000/mm^3; and white count of 6,700/mm^3, with 42% segmented neutrophils, 2% band neutrophils, 37% lymphocytes, 6% monocytes, 4% eosinophils, occasional basophils, and 9% atypical lymphocytes. Many lymphocytes (62.8%) bore surface immunoglobulin and were considered to be B cells. Immunoglobulin levels were as follows: IgM, 144 mg/dL; IgA, 179 mg/dL; IgG, 1,190 mg/dL, and IgE, 19.5 U/mL; all these values are normal in our laboratory. His blood was type O, Rh+; isohemagglutinin levels (anti-A and anti-B) were normal. Total hemolytic complement was normal at 84 CH_{50} U/mL. Bone marrow aspirate and biopsy were normocellular with a mild decrease in megakaryocytes. Lymphocyte transformation to the mitogens, phytohemagglutinin, pokeweed mitogen, and concanavalin A were all normal. Cultures of bone marrow for mycobacteria, fungi, anaerobic, and aerobic bacteria were all negative. The chest radiograph was normal. A radiograph of the sinuses showed mild increased soft tissue density over both maxillary sinuses consistent with minimal thickening of the mucoperiosteal membranes secondary to sinusitis. No air fluid levels were demonstrated and the remaining paranasal sinuses were normal.

Results of neutrophil activity profiles

Laboratory studies of PMN oxidative metabolism and motility were carried out in both cases. Capacity of PMNs to generate superoxide anion was determined using an enzyme immunoassay microplate reader in a modification of a procedure described previously.[6] Random migration and chemotaxis were evaluated in agarose petri dishes.[7] The synthetic peptide, *N*-formyl-methionyl-phenylalanine, was used as a chemoattractant.

In Case 1, the unstimulated nitroblue tetrazolium reduction (NBT) test was normal with 4% positive neutrophils. The neutrophil activity profile showed a mild reduction of random migration (7 migration units; normal, 12–24) (*Table 1*) and marked reduction of oxidative metabolism (20.7 nanomoles superoxide anion/10^6 neutrophils; normal, 40–60) (*Fig. 1*). Stimulated migration (chemotaxis) was within the normal range (35.3 units; normal, 31–54) (*Table 1*).

In Case 2, the unstimulated NBT test was

normal with 20% positive neutrophils. The neutrophil activity profile revealed poor cell adherence and markedly reduced random migration of 4.5 migration units (*Table 1*). Stimulated migration was normal at 32.7 chemotaxis units. Oxidative metabolism was also normal at 48 nanomoles superoxide anion/10^6 neutrophils (*Fig. 1*).

Comment

The first patient (Case 1) showed impaired oxidative metabolism with normal neutrophil motility. This functional defect, which may impair a person's ability to resist infection, could conceivably have been either of primary origin or drug-induced, as this patient was on prednisone at the time of testing. The NBT test was normal, however, this test is not quantitative and is generally abnormal only when there is a total absence of oxidative function, as in chronic granulomatous disease. The positivity of an NBT test also indicates that phagocytosis is normal. The condition seen in the first patient may have responded to vitamin C therapy. No follow-up data are available for this patient.

The second patient (Case 2) demonstrated normal oxidative metabolism, but neutrophil adherence and motility were impaired. This patient had frequent infections and had been told that he had a fatal disease, possibly chronic granulomatous disease. Our studies clearly show that his problem was of a less severe nature and involved cellular motility rather than oxidative metabolism. This is qualitatively the defect seen in the lazy-leukocyte syndrome, although the precise molecular defect in that syndrome has not been identified. Our patient's condition differed from the lazy-leukocyte syndrome in that he showed normal numbers of circulating neutrophils rather than severe neutropenia. Although no treatment is available for this patient's condition at present, reassurance as to the mild nature of the disease can be beneficial.

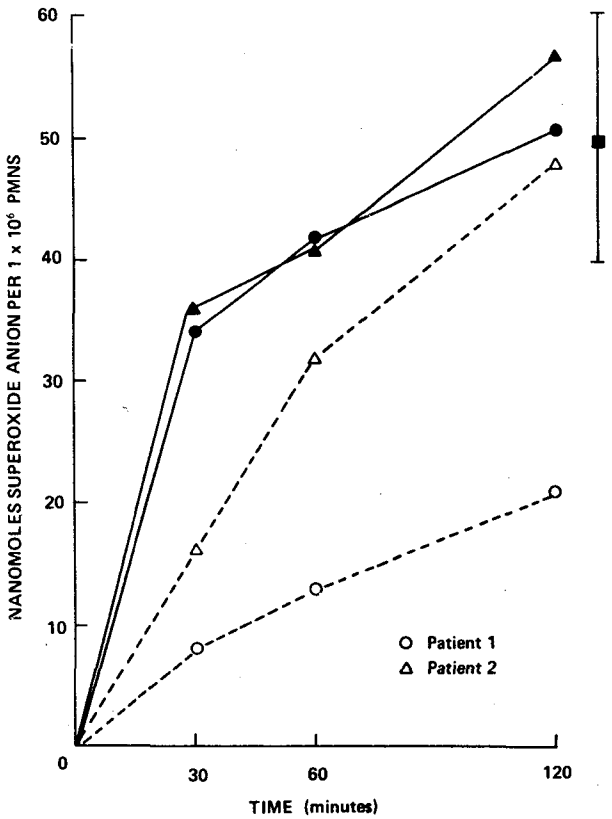

Fig. 1. Results of oxidative metabolism. Polymorphonuclear leukocytes (PMNS) from patient 1 (open circles), patient 2 (open triangles), and two normal individuals (closed circles and triangles) were stimulated with phorbol myristate acetate and monitored for superoxide anion production. Mean nanomoles superoxide anion ±2 S.D. accumulated after 120 minutes from PMNS of 10 normal individuals are indicated by the closed square and brackets.

Review

Physiology of neutrophils

Random motility and chemotaxis. Neutrophils are rapidly mobile cells, capable of moving quickly from one body compartment to another.[8] Although the exact mechanism of cell movement is unclear, contractile proteins, including actin and myosin, are undoubtedly of prime importance. Other more recently described structural and regulatory proteins include actin-binding protein, profilin, acumentin, and gelsolin.[9,10] Neutrophil movement occurs both randomly as well as in response to chemotactic stimuli. Chemotactic agents include complement cleavage products, lymphokines, leukotrienes, bacteria-derived peptides, and proteins involved in the kinin and coagulation pathway.[11] The manner by which a neutrophil translates a gradient of chemotactic activity into directed migration is extremely complex and is thought to involve binding of chemotactic agents by specific surface receptors, rapid changes in the transmembrane potential, changes in cyclic nucleotides, mobilization of calcium stores, altered phospholipid metabolism, and finally, reorganization of the cytoskeletal proteins.[9,10]

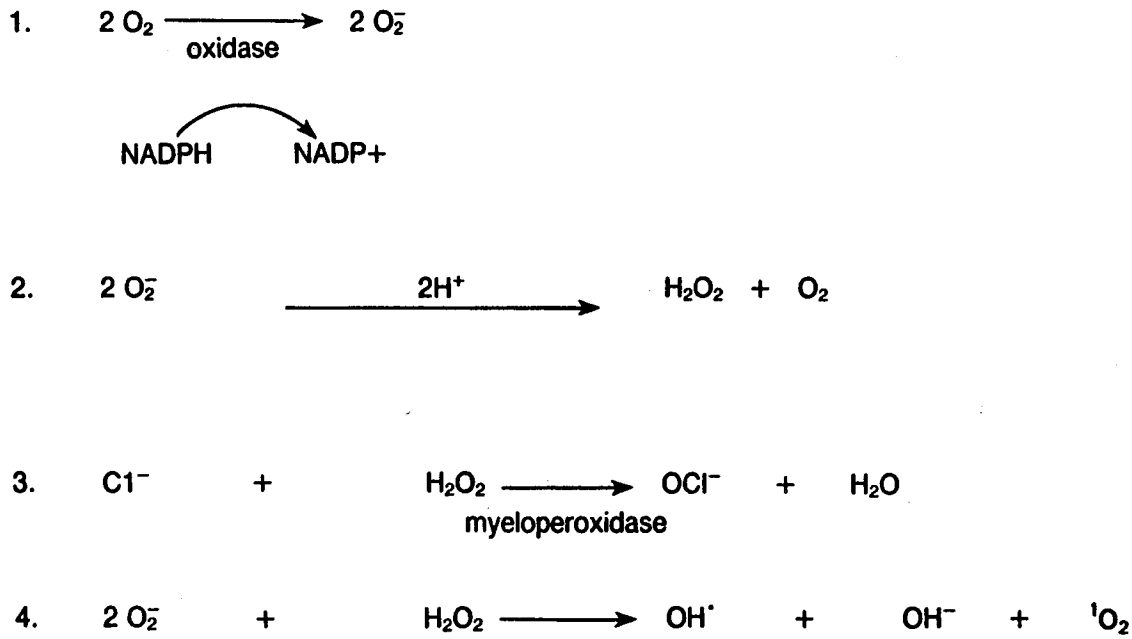

Fig. 2. Oxidative metabolism of the respiratory burst. Superoxide anion is the first reactant to be formed in the respiratory burst (*1*) followed by hydrogen peroxide (*2*). Myeloperoxidase catalyzes the reaction of hydrogen peroxide with chloride ion to form the bactericidal hypochlorous anion (*3*). Superoxide anion and hydrogen peroxide may interact to form hydroxyl radical, hydroxyl anion, and singlet oxygen (*4*).

As neutrophils migrate under the influence of chemotactic agents, they may encounter particulate cellular debris or bacterial organisms. The leading edge of the neutrophil consists of a relatively organelle-free structure, variously described as a pseudopod or lamellipod. When the advancing cell process encounters a particle that is recognized and can be ingested, the pseudopod flows around the object as increasing numbers of binding sites between the particle and receptors on the PMN surface are formed. Binding sites continue to form until the pseudopod fuses at the distal pole of the particle, closing in a manner that has been described as a "zipper-like" process.[10,12]

Cytoplasmic granules. Migration to a site of inflammation and interaction of the PMN with opsonized particles set in motion the biochemical machinery that partly resides in the neutrophil granules. The neutrophil contains two types of granules which are synthesized in a sequential fashion during maturation of the cell in the bone marrow. Primary or azurophilic granules are synthesized during the mitotic phase of neutrophil maturation. Secondary or specific granules are produced during the postmitotic phase and quantitatively represent the predominant granule type.[13] Partial purification of neutrophil granules by sucrose density gradient centrifugation following cell disruption has provided the opportunity to define the constituents of each type of granule.[14–16] The primary granules are similar to lysosomes in that they contain a number of acid hydrolases (including *N*-acetyl-beta-glucosaminidase, cathepsin B, cathepsin D, beta glucuronidase, beta glycerophosphatase, and alpha mannosidase) and serine proteases (elastase, cathepsin G, and collagenase). Myeloperoxidase is considered a marker enzyme for primary granules. Lysozyme, another bactericidal enzyme, is contained in both the primary and secondary granules. This enzyme can digest glycoprotein debris within the cell and can also hydrolyze the mucopolysaccharide portion of some bacterial cell walls.[17] In addition, the secondary granules contain vitamin B-12 binding proteins, cytochrome B, and a number of cationic proteins that can bind to the negatively charged surfaces of gram-negative bacteria and thereby inhibit bacterial growth. It has recently been proposed that the cationic proteins may act in a manner analogous to the antibiotic polymyxin B.[18,19] Finally, the secondary granules contain all of the neutrophil's lactoferrin. This molecule chelates iron and may

possess bacteriostatic activity by depriving the microorganisms of a necessary nutrient for growth. It has also been proposed that the lactoferrin-iron complex may enhance hydroxyl radical formation from superoxide anion and hydrogen peroxide.[20]

Secretion of granule contents. The manner in which the neutrophil granule components are released to perform their antimicrobial and digestive functions has been carefully studied using a combination of ultrastructural and biochemical methods. Some of the specific granules are thought to fuse with the plasma membrane and release their contents into the extracellular environment.[12] Neutrophils may therefore be thought of as secretory, as well as phagocytic cells, and may amplify and localize the inflammatory response through this secretory activity. Fusion of specific granules with the plasma membrane is also thought to achieve translocation of cytochrome B from an intracellular pool to the plasma membrane,[21] thereby completing the electron transport system that is required for the production of oxygen containing antibacterial agents. The fusion of both types of granules with phagocytic vacuoles has been carefully studied using electron microscopy and cytochemistry.[12,22,23] It appears that secondary granules release their contents into phagocytic vacuoles first, allowing the constituents to act while the pH of the vacuole is near neutral, or close to the optimal pH range. Following a short lag phase, during which the pH in the vacuole falls to about 4.0, the primary granules discharge their contents into what may then be considered a secondary lysosome, in which enzymes with an optimal pH in the acid range are able to carry out their activities.

Microbicidal processes. Oxidative bactericidal processes are perhaps the most potent weapons employed by the neutrophil. The importance of the oxygen-dependent bactericidal mechanisms is emphasized first by the finding that microbial killing is markedly reduced under anaerobic conditions,[24,25] and second, by the fact that inherited defects in the oxidative machinery lead to impaired microbial killing by neutrophils and severe recurrent infections in the affected patients. Within seconds after phagocytic stimulation, oxygen consumption by the neutrophil increases dramatically in what has been called a "respiratory burst."[10,17,26,27] The basic reactions are summarized in *Figure 2*. Reducing equivalents in the form NADPH are provided by the hexose-monophosphate shunt pathway. Glucose-6-phosphate dehydrogenase is of prime importance in this metabolic pathway. A membrane-associated NADPH oxidase complex, which is thought to contain a flavoprotein subunit as well as cytochrome B, catalyzes the formation of superoxide anion from molecular oxygen. Superoxide dismutase then catalyzes the formation of hydrogen peroxide which itself can combine with superoxide to form hydroxyl radicals and singlet oxygen. Finally, myeloperoxidase can use the hydrogen peroxide to produce highly reactive halide-containing species such as hypochlorite. These various oxygen-containing species are highly reactive and thought to possess potent bactericidal activity.[28]

Table 2. Intrinsic neutrophil defects

Granule defects
Myeloperoxidase deficiency
Lactoferrin deficiency
Chediak-Higashi syndrome
Metabolic disorders
Chronic graulomatous disease and related disorders
G-6-phosphate dehydrogenase deficiency
Motility disorders
Lazy-leukocyte syndrome
Neutrophil actin dysfunction
Hyperimmunoglobulin E syndromes
Transient chemotactic disorder of neonates
Other familial chemotactic defects
Miscellaneous disorders
Plasmalemma glycoprotein deficiencies
Juvenile periodontitis

Neutrophil dysfunction

Extrinsic factors. Disorders of neutrophil function can be separated generally into those secondary to extrinsic factors and those apparently related to intrinsic cell defects. Extrinsic factors which can cause neutrophil dysfunction include ethanol[29] and other pharmacologic agents,[8,30] hemodialysis,[31] and extensive burns.[32,33] In addition, bone marrow involvement by neoplastic diseases can severely restrict the quantitative response of neutrophils to microbial invasion. Also included in this broad category are neutrophil dysfunctions related to systemic diseases such as diabetes mellitus,[34] sarcoidosis,[35]

cirrhotic liver disease,[8] uremia,[31] and Hodgkin's disease.[36]

Intrinsic defects. Intrinsic neutrophil defects may primarily affect either cell migration or metabolic responses. In some cases, multiple defects involving both functional categories are evident. *Table 2* outlines some of the various intrinsic functional disorders of neutrophils.

In a small group of patients with abnormal chemotaxis, phagocytosis, and cell adhesion, biochemical analysis has revealed either decreased or absent expression of glycoproteins recognized by monoclonal antibodies. These glycoproteins have been designated Mo1 and LFA-1.[37–39] Recent studies have demonstrated that LFA-1 and Mo1 are important in the adhesion of neutrophils to endothelial cells and in the phagocytosis of opsonized particles. Mo1 may also function as a complement receptor and may therefore participate in the early steps of cell activation. Patients with deficiencies of these glycoproteins suffer from recurrent pyogenic infections, which may become apparent in the first few weeks of life. In those patients with severe disease, death may occur at less than five years of age.

Patients with localized juvenile periodontitis may represent another group with deficient cell surface proteins.[40] This disease is characterized by unusually severe periodontal disease with an early onset that is usually restricted to the area of the first molars and incisors. It is a rare disorder and has a tendency to cluster in families. Recent biochemical studies have demonstrated decreased numbers of receptors for both *N*-formyl-methionyl-leucyl-phenylalanine, a structural analog of a number of bacterial chemotactic agents, and for C5a. As might be predicted, these patients have significantly reduced chemotaxis.

Another well-defined biochemical deficiency is the partial or complete absence of neutrophil myeloperoxidase. This disorder is not as uncommon as once thought and may in fact represent one of the most common intrinsic neutrophil disorders. In a large study involving the screening of 60,000 patients, 28 were found to be myeloperoxidase deficient, with approximately one half of those having a complete loss of neutrophil myeloperoxidase activity.[41] Only two patients in that study had a history of persistent or severe infections. One patient died with *Candida* pneumonia and the second had persistent *Bacteroides fragilis* bacteremia. In contrast, most of the myeloperoxidase deficient patients were free of infectious symptomatology. *Candida* seems to be a frequent cause of infection in those myeloperoxidase-deficient patients that have infections, particularly when their deficiency is complicated by diabetes mellitus. In vitro studies have demonstrated that killing of *Candida albicans* by neutrophils from patients with complete myeloperoxidase deficiency averages less than 10% of control values.

Chronic granulomatous disease, another genetically inherited disorder, may represent a heterogeneous group of molecular defects that share in common a significantly decreased respiratory burst following phagocytosis.[42] Patients with chronic granulomatous disease usually present in infancy and suffer particularly from infection by catalase-positive microorganisms. *Staphylococcus aureus* is the most frequent pathogen, and patients frequently present with suppurative lymphadenopathy, hepatosplenomegaly secondary to abscesses, pneumonia, and a variety of dermatologic infections including impetigo and subcutaneous abscesses.[42,43] In some patients, there is deficient neutrophil membrane depolarization,[1] suggesting a defect in the early steps of stimulus-response coupling by neutrophils. Some patients with a chronic granulomatous disease-like illness have complete glucose-6-phosphate dehydrogenase deficiency,[1] resulting in a marked decrease in NADPH generation, thereby causing low activity of the surface oxidase complex. Other forms of the disease are characterized by specific abnormalities of the surface oxidase itself. In some, there is a decreased affinity of the oxidase for NADPH,[44] and in others, the cytochrome b component is lacking.[45] The heterogeneous molecular defects in chronic granulomatous disease help to explain the variable inheritance pattern. The most frequent form of the disease is X linked and therefore primarily affects males, but an autosomal recessive pattern has also been reported.[45]

Boxer et al[46] described an infant with uncontrolled chronic *Pseudomonas* infection, elevated neutrophil counts, and normal oxidative metabolism. There was, however, abnormal neutrophil migration and markedly decreased phagocytosis of opsonized particles. Biochemical analysis showed altered polymerization of the microfilament protein, actin. Ultrastructural examination of the patient's neutrophils revealed fewer microfilament-rich pseudopodia than normal. This defect has not been reported in other patients; however, the case does serve to emphasize the

important role of actin in the motile functions of neutrophils.

Another defect in neutrophil migration has been called the lazy-leukocyte syndrome. The syndrome is rare, with less than 10 cases reported in the English literature to date.[47–50] It was first described in 1971 in two children with recurrent stomatitis, otitis, gingivitis, and severe peripheral neutropenia.[47] In contrast to the peripheral blood, the bone marrow contained adequate numbers of neutrophils and neutrophil precursors, with normal neutrophil maturation. However, piromen, a bacterial product that normally stimulates emigration of neutrophils from the bone marrow, failed to cause an increase in the peripheral neutrophil count in the involved patients. In vitro studies revealed decreased chemotaxis and random migration with normal phagocytosis and bactericidal activity. This disorder is clinically mild. Infections tend not to be severe and the affected children grow and develop normally. The molecular basis of the neutrophil defect in the lazy-leukocyte syndrome is uncertain. Some investigators have proposed that the defect may somehow involve the interaction between microtubules and the cell surface.[49]

The Chediak-Higashi syndrome is quite rare and has an autosomal recessive mode of inheritance. Patients present with oculocutaneous pigmentary dilution, photophobia, and increased susceptibility to infections, most commonly caused by *Staphylococcus aureus*.[51–53] Diagnosis is based largely on the presence of large, lysosome-like, cytoplasmic inclusions in both neutrophils and lymphocytes, as well as in various tissue cells.[51–53] The patients usually progress to an accelerated lymphoma-like phase, characterized by generalized lymphohistiocytic infiltrates producing hepatosplenomegaly and lymphadenopathy accompanied by pancytopenia and fever. Laboratory studies of neutrophil function reveal defective degranulation, impaired chemotaxis, and reduced bactericidal activity. The exact basis of the neutrophil defect in the Chediak-Higashi syndrome has yet to be satisfactorily defined.

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