Review Articles

Advances in Immunology

IMMUNODEFICIENCY DISEASES CAUSED BY DEFECTS IN PHAGOCYTES

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P RIMARY phagocytic defects must be included in the differential diagnosis of recurrent infection and fever in a child and occasionally in an adult. Early diagnosis is essential, because manifestations of infection are usually blunted and rapid intervention can be lifesaving. In general, patients are identified at a young age on the basis of their susceptibility to normally nonpathogenic bacteria or fungi. In some cases, the infectious agents point to the disorder (Table 1): catalase-positive microorganisms and aspergillosis species are characteristic of chronic granulomatous disease,[1] and atypical mycobacteria suggest a defect in the interferon-γ–interleukin-12 axis.[2] These bacterial infections contrast with the viral and candida infections in deficiencies of T cells. Also suggestive is the failure of an infection to resolve with conventional treatment.[3] Other characteristic findings include recurrent infections of the lungs, liver, and bone; aphthous ulcers; severe gingivitis; and in some disorders, periodontitis. Sepsis or meningitis is rare, but lymphadenopathy and hepatosplenomegaly are common.

Nearly all primary defects of phagocytes result from a mutation that affects the innate immune system. For example, leukocyte adhesion deficiency type 1 results from the loss of an adhesion protein on neutrophils, which causes leukocytosis owing to an impaired ability of neutrophils to exit the circulation and travel to sites of infection.[4] Identification of the mutations underlying primary phagocytic disorders has provided exciting revelations connecting molecular findings to the clinical aspects of these diseases.

CONGENITAL NEUTROPENIAS

A defect in the life cycle of neutrophils can compromise host defenses.[5] Severe neutropenia, defined

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as an absolute neutrophil count of less than 500 cells per cubic millimeter,[6-8] suppresses inflammation and increases susceptibility to recurrent and severe bacterial and fungal infections. Descriptions of patients with infections and neutropenia appeared early this century,[9] but in 1930, Roberts and Kracke showed that neutropenia can precede infection.[10] Soon thereafter, neutropenias were subdivided into asymptomatic neutropenias and those associated with bone marrow insufficiency.[11]

Cyclic Neutropenia

Cyclic neutropenia is an autosomal dominant disorder in which cyclic hematopoiesis causes intervals of neutropenia and susceptibility to opportunistic infection.[6,7] Recurrent, very severe neutropenia (an absolute neutrophil count of less than 200 cells per cubic millimeter) that lasts 3 to 6 days of every 21-day period is typical. In about 30 percent of patients with cyclic neutropenia, however, the cycles range from 14 to 36 days.[8] Patients are usually asymptomatic, but during the period of severe neutropenia, aphthous ulcers, gingivitis, stomatitis, and cellulitis may develop, and death from overwhelming infection occurs in about 10 percent of patients.[8,12] Abdominal pain must be assessed aggressively because of the high frequency of clostridium infections during the period of severe neutropenia.[12] During the periods of neutropenia the bone marrow shows lack of maturation of granulocyte precursors beyond myelocytes; moreover, there is myeloid hyperplasia during the remainder of the cycle. Occasionally, there is a reduction in the severity of neutropenia and the accompanying infections over time.[8,12]

Mutations in the neutrophil elastase gene (*ELA2*) have been identified in patients with cyclic neutropenia.[13] Neutrophil elastase is released from neutrophils during inflammation and causes the destruction of tissues.[13,14] The mutations affect the catalytic site of the enzyme, which can lead to the failure of inhibitors to bind and thus inactivate elastase.[15] Mice with an inactivated neutrophil elastase gene have normal numbers of neutrophils, but the ability of these cells to kill pathogens is impaired.[16] Thus, the link between neutrophil elastase and the cyclic changes in the levels of neutrophils is unknown.

Mathematical models suggest that granulocyte colony-stimulating factor may modulate the control of both the numbers of circulating neutrophils and the development of hematopoietic stem cells into granulocyte precursors.[17] Defects in granulocyte colony-stimulating factor signaling could destabilize normal steady-state conditions and increase the numbers of

TABLE 1. IMMUNODEFICIENCY DISEASES CAUSED BY DEFECTS IN PHAGOCYTES.

DISEASE	MOLECULAR OR GENETIC DEFECT	PATHOGENIC ORGANISMS AND SITES AFFECTED	CLINICAL PRESENTATION
Severe chronic neutropenia			
Cyclic neutropenia	Mutation in *ELA2,* encoding neutrophil elastase	Episodic bacterial infections, including those due to *Clostridium perfringens;* aphthous ulcers; gingivitis; stomatitis; cellulitis	21-Day oscillations in neutrophil, monocyte, platelet, and lymphocyte counts
Severe congenital neutropenia	Unknown	*Staphylococcus aureus, Burkholderia aeruginosa;* cellulitis, perirectal abscess, stomatitis, meningitis	Developmental arrest of bone marrow myeloid cells at the promyelocyte stage; usually responsive to treatment with granulocyte colony-stimulating factor; increased risk of acute myelogenous leukemia and the myelodysplastic syndrome in some forms
Shwachman–Diamond syndrome	Unknown	Infections involving the lungs, bone, skin, urinary tract	Cyclic or intermittent neutropenia, pancytopenia; associated skeletal abnormalities; pancreatic insufficiency; recurrent infections of the sinuses, lungs, bones, skin, and urinary tract; increased risk of aplasia, myelodysplasia, and leukemia
Leukocyte adhesion deficiency			
Type 1	CD18	Gram-negative enteric bacteria, *S. aureus,* candida species, aspergillus species	Recurrent infections of skin, soft tissues, and respiratory and gastrointestinal tracts; periodontal disease; delayed separation of the umbilical cord
Type 2	Carbohydrate fucosylation	Gram-negative enteric bacteria, *S. aureus,* candida species, aspergillus species	Recurrent infections of skin, soft tissues, and respiratory and gastrointestinal tracts; periodontal disease; delayed separation of the umbilical cord; growth retardation; dysmorphic features; neurologic deficits
Rac2 deficiency	Deficiency of Rac2	Not reported	Recurrent perirectal abscesses, poor wound healing, absence of pus at sites of infection, leukocytosis, and neutrophilia
Interferon-γ and interleukin-12 defects	Interferon-γ–receptor ligand-binding chain, interferon-γ–receptor signaling chain, interleukin-12–receptor $\beta1$ chain, interleukin-12 p40 deficiency	Bacille Calmette–Guérin, *Mycobacterium avium* complex, *M. fortuitum, M. chelonae, M. smegmatis,* salmonella species	Infection with intracellular microorganisms with severe mycobacterial disease; onset in infancy, dissemination, and failure to form granulomas seen with autosomal recessive interferon-γ–receptor defects; later-onset osteomyelitis is associated with autosomal dominant interferon-γ–receptor defects
Chronic granulomatous disease of childhood	gp91^{phox} (in X-linked chronic granulomatous disease) p47^{phox} p67^{phox} p22^{phox}	Catalase-positive microorganisms: *S. aureus, B. cepacia,* aspergillus species, nocardia species, *Serratia marcescens*	Abscess formation in the lungs, liver, brain, and bone; soft-tissue infection; gastrointestinal and urogenital obstruction from granulomas
Myeloperoxidase deficiency	Defects in *MPO* at chromosome 17, q11–21, q22–24, q21.3–23	Not usually associated with clinical disease	Associated with disseminated candidiasis in patients with diabetes mellitus
Chédiak–Higashi syndrome	Mutation in LYST, encoding a cytoplasmic protein involved in protein transport	*S. aureus,* beta-hemolytic streptococcus	Partial ocular and cutaneous albinism, peripheral neuropathy, recurrent bacterial infections, easy bruising, mild mental retardation, severe periodontal disease
Neutrophil-specific granule deficiency	*C/EBPε,* encoding a transcription factor	*S. aureus, S. epidermidis,* enteric bacteria	Recurrent infections of skin and lungs, poor healing, bleeding diatheses

circulating lymphocytes, reticulocytes, and platelets, as occurs in cyclic neutropenia.[17]

Severe Congenital Neutropenia

Severe congenital neutropenia is characterized by severe neutropenia (an absolute neutrophil count of less than 500 cells per cubic millimeter), recurrent bacterial infections, and failure of myeloid cells to mature from promyelocytes to myelocytes.[6-8] The disease begins during the first year of life, and its infectious complications include cellulitis, perirectal abscess,

peritonitis, stomatitis, and meningitis, commonly as a result of infections with *Staphylococcus aureus* and *Burkholderia aeruginosa.*[8] The numbers of circulating monocytes and eosinophils are often increased.[8] Despite having increased plasma levels of granulocyte colony-stimulating factor, nearly all patients have a response to pharmacologic doses of recombinant granulocyte colony-stimulating factor (filgrastim); neutrophil counts rise, infection rates fall, and mortality is reduced.[18]

Although severe congenital neutropenia was orig-

inally described by Kostmann in 1956 as an autosomal recessive disease, the underlying mutation is unknown. Some patients have acquired mutations in myeloid lineages,[19] and these patients are at risk for the myelodysplastic syndrome and acute myelogenous leukemia.[19] In about 10 percent of patients, a heterozygous mutation inhibits the signaling function of the receptor for granulocyte colony-stimulating factor.[19]

The Shwachman–Diamond Syndrome

First described in 1964, the Shwachman–Diamond syndrome is a rare autosomal recessive disorder that is characterized by exocrine pancreatic insufficiency, skeletal abnormalities, bone marrow dysfunction, and recurrent infections.[8] Neutropenia, either cyclic or intermittent, occurs in all patients, and 10 to 25 percent of patients also have pancytopenia.[20] Recurrent infections begin during the first year of life and commonly involve the sinuses, lungs, bones, skin, and urinary tract.[8] These patients have an increased risk of bone marrow aplasia, myelodysplasia, and leukemia.[21] The average life expectancy is 35 years, but it is less in patients with pancytopenia or malignant transformation.[22]

Treatment of Congenital Neutropenias

In almost all patients with severe chronic neutropenia, treatment with filgrastim results in significantly fewer infections.[6,7] Concern that filgrastim could fuel the development of acute myelogenous leukemia in these patients has not been borne out,[6,7] and the annual rate of malignant conversion has not increased with its use.[6,7] Malignant conversion is also not seen with prolonged use of filgrastim in patients with cyclic or idiopathic neutropenia.[6,7]

DEFECTS OF ADHESION

In 1980 a patient was described who had an elevated neutrophil count, recurrent infections, and few neutrophils in inflammatory foci.[23] The boy had a defect in neutrophil adherence, and his neutrophils lacked a 110-kd surface glycoprotein. Subsequently, it was recognized that the process of aggregation and attachment of neutrophils to endothelial surfaces was mediated by a group of molecules called integrins and selectins and that these molecules are essential for a normal inflammatory response.[24]

Several molecular defects of leukocyte adhesion cause recurrent life-threatening infections. Leukocyte adhesion deficiency type 1 is an autosomal recessive disorder resulting from a lack of β_2 integrin adhesion molecules on neutrophils.[25] There are three β_2 integrins, which have different α chains but a common β chain, called CD18.[26] Defects in CD18 account for the loss of β_2 integrin and the clinical findings in patients with the disorder.[25]

Neutrophils in patients with leukocyte adhesion deficiency type 1 are unable to aggregate (Fig. 1A and 1B).[27] Also, they do not bind to intercellular adhesion molecules on endothelial cells, a step that is necessary for their egress from the vasculature and transport to sites of inflammation.[28] As a result, even when there is no infection, the neutrophil count is about twice the normal level.[28]

Clinical features suggesting leukocyte adhesion deficiency type 1 include a history of delayed separation of the umbilical cord[28]; severe periodontitis, often resulting in early tooth decay (Fig. 1C)[28]; and recurrent infections of the oral and genital mucosa, skin, and intestinal and respiratory tracts.[28] Infecting pathogens include gram-negative enteric bacteria, *S. aureus,* candida species, and aspergillus species.[25,26,28,29] Infected foci contain few neutrophils and heal slowly, with enlarging borders and dysplastic scars.

Patients with leukocyte adhesion deficiency type 1 who have no detectable CD18 have the worst prognosis, and most die by the age of 10 years.[4] Patients whose levels of CD18 are 1 to 10 percent of the normal levels may live 40 years or longer, and some may not receive a specific diagnosis until they are in their late teens.[4]

The second type of leukocyte adhesion deficiency is a defect of carbohydrate fucosylation and is associated with growth retardation, dysmorphic features, and neurologic deficits.[30-32] The loss of $\alpha 1,2$-, $\alpha 1,3$- and $\alpha 1,6$-linked fucose groups in a variety of carbohydrates suggests that patients with leukocyte adhesion deficiency type 2 have a general defect in the generation or transport of guanosine diphosphate–L-fucose.[33] These patients lack sialyl-Lewis^X, a ligand for the selectin family, and in these patients, there is no fucosylation of other glycoconjugates that are required for interactions with P-selectins and E-selectins on endothelial cells.[32] The genetic defect has not been determined, however. Treatment with oral fucose has reduced the frequency of infections and fevers.[34]

Deficiency of ras-related C3 botulinum toxin substrate (Rac 2), the predominant GTPase in neutrophils, was reported in a five-week-old boy with typical signs of leukocyte adhesion deficiency.[35] The baby's neutrophils exhibited abnormal chemotaxis and secretion of primary granules and defective generation of superoxide in response to formyl peptides.[35] The respiratory burst in response to phorbol myristate acetate was normal, affirming the presence of functional NADPH oxidase. Rac 2 is integral to the function of the actin cytoskeleton. Rac 2 deficiency results in the inability of neutrophils to move normally in response to bacterial peptides.[35]

DEFECTS OF SIGNALING

For nearly 100 years, an attenuated strain of *Mycobacterium tuberculosis,* bacille Calmette–Guérin (BCG), has been used to immunize newborns in many European countries. About 30 years ago several cases of fatal BCG infection were reported.[36]
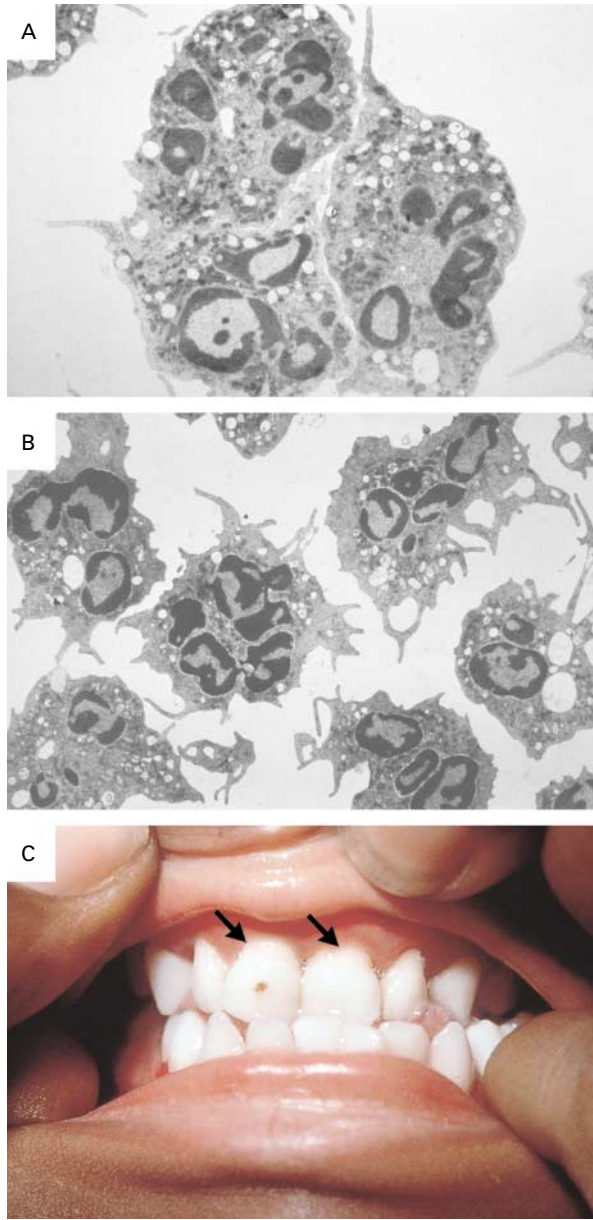
Figure 1. Features of Leukocyte Adhesion Deficiency Type 1.
In Panel A, normal neutrophils aggregate readily in response to phorbol myristate acetate (100 ng per milliliter) (×500), whereas in Panel B, neutrophils from a patient with leukocyte adhesion deficiency type 1 fail to aggregate (×500). Patients with leukocyte adhesion deficiency type 1 have periodontitis (arrows in Panel C) with early tooth loss as part of a constellation of recurrent infections involving the gastrointestinal, urogenital, and respiratory tracts. Panels A and B reprinted from Rotrosen and Gallin[27] with the permission of the publisher.

Half the affected infants had a profound deficiency of T cells as a result of severe combined immunodeficiency, but the rest had no obvious immunologic defect. Their problem was clarified by the study of several related children from a small, isolated fishing village on the island of Malta[37] who had fatal infections with atypical mycobacteria that were not considered to be pathogenic. The susceptibility gene in this kindred was mapped to chromosome 6 at the precise site where the receptor for interferon-γ is encoded.[37]

The interferon-γ–interleukin-12 axis is critical for defenses against intracellular microbes such as mycobacteria, salmonella, and listeria (Fig. 2). Defects in the ligand-binding chain of the interferon-γ receptor, the signaling chain of the interferon-γ receptor, the interleukin-12 receptor, or interleukin-12 itself increase susceptibility to mycobacterial infection.[2] Variations in the clinical manifestations and severity of disease and the presence or absence of a response to treatment with interferon gamma reflect the extent of the disruption of the interferon-γ–interleukin-12 axis.[2]

The presence of a pathogen triggers the production of interleukin-12 by dendritic cells and macrophages, which in turn induces the secretion of interferon-γ by T cells and natural killer cells (Fig. 2).[38] Interferon-γ activates macrophages and neutrophils, causing them to produce tumor necrosis factor α and activate NADPH oxidase, which promotes killing of the pathogen by increasing the production of hydrogen peroxide.[38] Interleukin-12 is also part of a feedback control mechanism. It induces T cells to produce interleukin-10, which suppresses the proliferation of T cells and the production of interleukin-12 and interferon-γ.[39,40]

The interferon-γ receptor consists of a ligand-binding chain and a signaling chain (also called the R1 and R2 chains, respectively).[2] Binding of interferon-γ to the ligand-binding chain of the receptor causes it to link up with another such chain and leads to the aggregation of two interferon-γ–receptor signaling chains.[2] Mutations have been identified in the genes for both chains of this receptor, with both autosomal recessive and autosomal dominant inheritance. Children with a mutation that causes complete loss of the ligand-binding chain have severe disease that begins in early infancy. The main features are disseminated atypical mycobacterial disease or fatal BCG infection after vaccination,[2,37,41] an inability to form granulomas (Fig. 2), and the absence of a response to high doses of interferon gamma. A mutation resulting in the partial loss of the ligand-binding chain of the interferon-γ receptor causes less severe disease, in which the capacity to form granulomas and responsiveness to high doses of interferon gamma are not lost.[42]

Children with a different mutation of the interferon-γ–receptor ligand-binding chain have milder disease; nontuberculous mycobacterial infections devel-
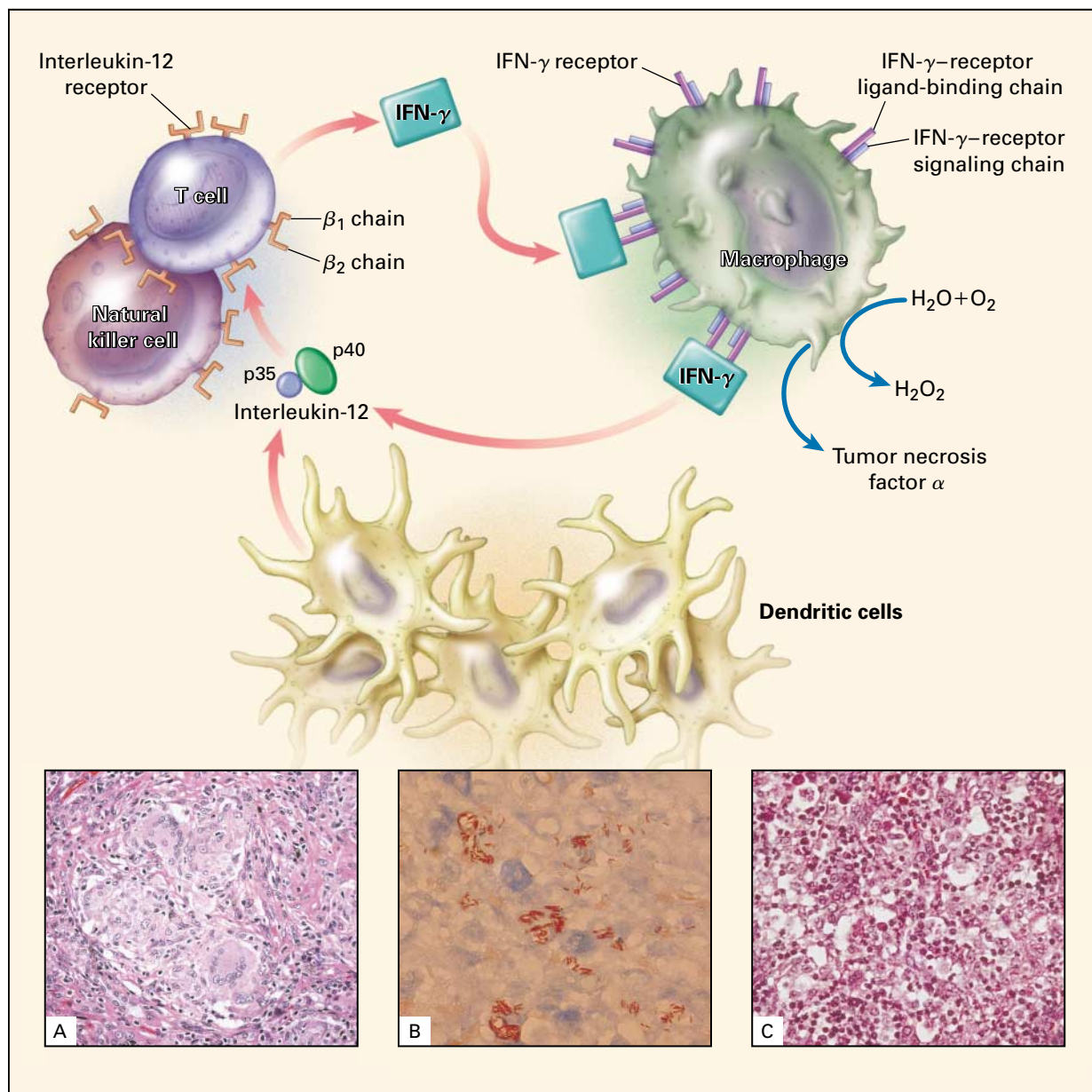
Figure 2. Interferon-γ–Interleukin-12 Signal-Transduction Cascade.

Interleukin-12, which is produced by macrophages and dendritic cells in response to the presence of a pathogen, binds to its receptors on T cells and natural killer cells, inducing the release of interferon-γ (IFN-γ). Monocytes and macrophages bind interferon-γ, resulting in the cross-linking of the interferon-γ receptor; activation of the cells, with the production of hydrogen peroxide (H_2O_2); and the synthesis and release of tumor necrosis factor α and interleukin-12 (dimer of subunits p35 and p40). Mutations resulting in increased susceptibility to nontuberculous mycobacteria have been identified in the genes for both ligand-binding chain and the signaling chain of the interferon-γ receptor, the β1 chain and the β2 chain of the interleukin-12 receptor (the β2 chain is the signal transducer), and the p40 subunit of interleukin-12. Panel A shows a resolving mycobacterial infection with normal granuloma formation in a lung-biopsy specimen from a patient with no known mutation in the interferon-γ–interleukin-12 axis (hematoxylin and eosin, ×20). Panel B shows a lung-biopsy specimen from a patient with an autosomal recessive mutation of the interferon-γ–receptor ligand-binding chain who was infected with nontuberculous mycobacteria (acid-fast Fite's stain, ×600). There are numerous mycobacteria (red) within macrophages (blue). Panel C shows a contiguous section of lung from the same patient in which there is no granuloma formation (hematoxylin and eosin, ×200).

op in early childhood rather than infancy, and they respond to treatment with interferon gamma.[2,43] Interestingly, in one report 13 of 16 patients with this mutation had nontuberculous mycobacterial osteomyelitis.[32]

A mutation in the gene for the receptor-signaling chain of interferon-γ that eliminates signaling also increases susceptibility to nontuberculous mycobacterial disease. The clinical presentation resembles that associated with the complete loss of the interferon-γ–receptor ligand-binding chain.[44]

Diminished production of interferon-γ as a result of abnormal regulation of interleukin-12 also increases susceptibility to disseminated nontuberculous mycobacterial or BCG infection.[2] The interleukin-12 receptor has two chains, β1 and β2. Both are required for high-affinity binding of interleukin-12; however, signal transduction is mediated by the β2 chain.[45] The clinical effect of a mutation in the gene encoding the β1 chain, which is associated with an increased susceptibility to nontuberculous mycobacterial disease and salmonella infections,[2,46,47] resembles that of a defect in the gene for the interferon-γ–receptor ligand-binding chain, and patients with this mutation have a response to interferon gamma therapy.[2]

Interleukin-12 also has two chains, a 35-kd and a 40-kd chain. A mutation in the gene for the 40-kd chain increases susceptibility to mycobacterial disease.[48] Patients with a mutation in the gene for this chain or the β1 chain of the interleukin-12 receptor can form mature granulomas, suggesting that they can produce interferon-γ in the absence of interleukin-12. As expected, patients with these mutations have a response to treatment with interferon gamma.[2]

DEFECTS OF INTRACELLULAR KILLING

The responses of phagocytes to pathogens include phagocytosis, proteolytic destruction within granules, and damage induced by hydroxyl radical, superoxide, and hydrogen peroxide generated by NADPH oxidase. Patients with defects in intracellular killing of microbes have increased susceptibilities to specific pathogenic bacteria and fungi that result in atypical and often muted inflammatory responses.

In 1954, Janeway and colleagues described children with elevated serum gamma globulin levels and recurrent infections,[49] some of whom were later shown to have chronic granulomatous disease. In 1957, four boys with hypergammaglobulinemia, recurrent infections of the lungs, lymph nodes, and skin, and granulomatous lesions were described.[50] An evaluation of phagocytic function by the available methods did not reveal any defects, and the disorder was named "fatal granulomatous disease of childhood." In 1967, a specific defect in the intracellular killing of bacteria was identified[51] and traced to the oxidative metabolism of phagocytes.[52]

The discovery of the protein components of the NADPH oxidase apparatus was the direct consequence of studies of neutrophils from patients with chronic granulomatous disease. These neutrophils were found to have defects in the generation of hydrogen peroxide and in NADPH oxidase function.[53-57] It became evident that chronic granulomatous disease is a heterogeneous disorder caused by defects in any one of the four subunits of NADPH oxidase,[58-68] the enzyme that initiates the process of forming hydrogen peroxide (Fig. 3).[69]

The most common form of chronic granulomatous disease (present in approximately 70 percent of patients) is X-linked and is due to a mutation in the gene for the phagocyte oxidase cytochrome glycoprotein of 91 kd ($gp91^{phox}$).[70] The second most common form is autosomal recessive and is due to a mutation in the gene for a cytosolic component of 47 kd ($p47^{phox}$).[70]

Chronic granulomatous disease is characterized by recurrent infections with catalase-positive microorganisms, which destroy their own hydrogen peroxide, including *S. aureus, Burkholderia cepacia,* aspergillus species, nocardia species, and *Serratia marcescens.*[1] Infections with catalase-negative organisms, such as *Streptococcus pneumoniae,* are rare. The clinical manifestations include recurrent or persistent infections of the soft tissues, lungs, and other organs, despite aggressive antibiotic therapy (Fig. 4).[1,38,70] The appearance of fever and clinical signs of infection may be delayed, requiring routine follow-up every four to six months. Magnetic resonance imaging of the chest in asymptomatic children often reveals early pneumonia. Severe, resistant facial acne and painful inflammation of the nares are common (Fig. 4A). Severe gingivitis (Fig. 4C) and aphthous ulcers are seen, but not periodontal disease (Fig. 1C), as is found in patients with leukocyte adhesion deficiency type 1. In addition, there is excessive formation of granulomas in all tissues. These granulomas, which can obstruct the genitourinary and gastrointestinal tracts,[71,72] are exquisitely responsive to short courses of corticosteroids (Fig. 4D).[72] Chronic granulomatous disease can easily be diagnosed by a nitroblue tetrazolium test (Fig. 5)[56] or by flow cytometry with dihydrorhodamine dye.[73]

Mycobacterial disease is uncommon in patients with chronic granulomatous disease,[74] but draining skin lesions and lymphadenopathy can occur at the site of BCG inoculation. Focal or miliary pulmonary disease may arise as a result of infection with nontuberculous mycobacteria, and it may be followed by pulmonary fibrosis.[75] Intracellular killing of mycobacteria is impaired in macrophages from patients with chronic granulomatous disease, demonstrating the essential role of NADPH oxidase in host defense against mycobacteria.[76]

Children with X-linked chronic granulomatous disease are more severely affected than children with
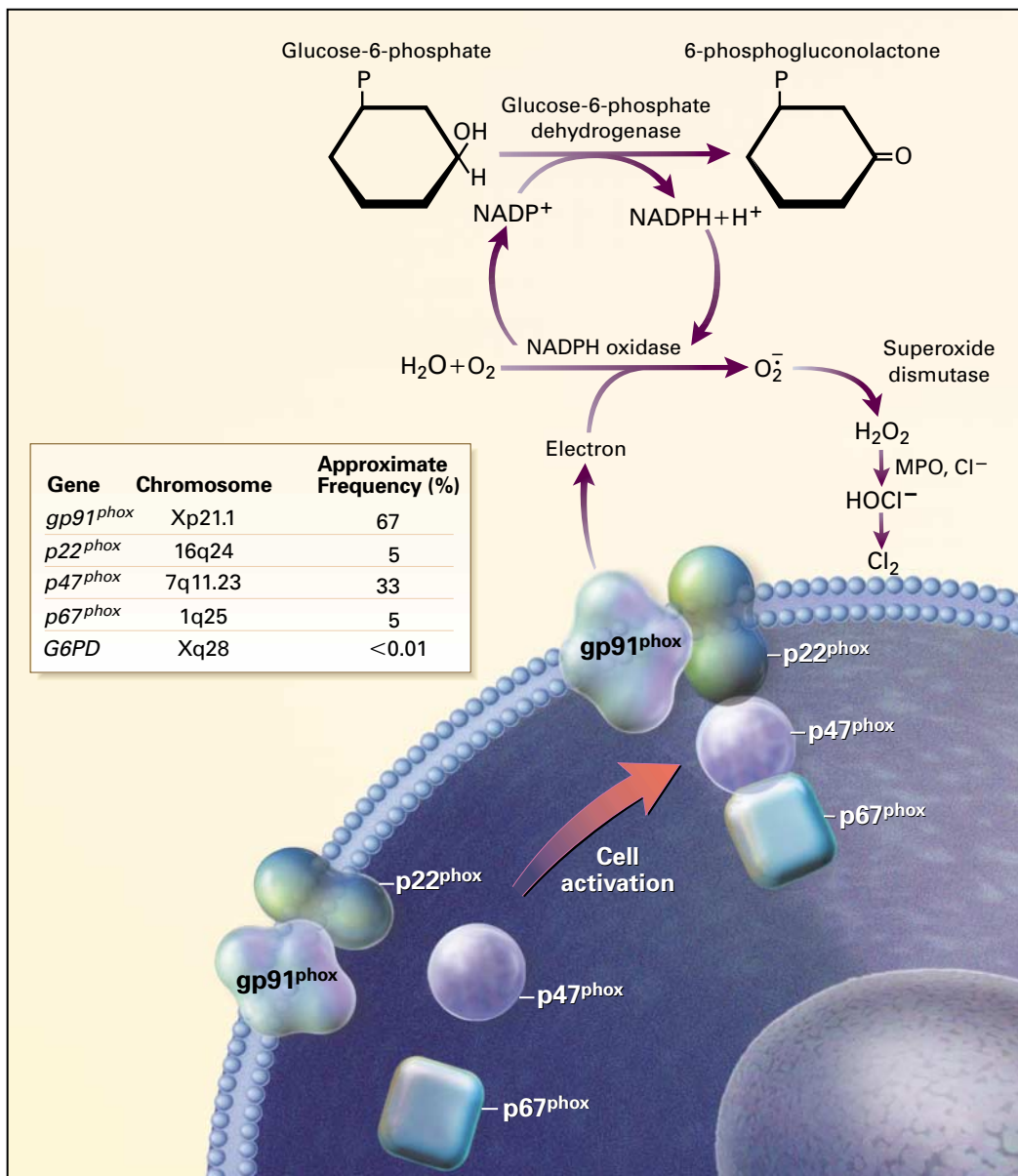
Figure 3. Relation among the Components of NADPH Oxidase That Are Affected in Patients with Chronic Granulomatous Disease.
The membrane-bound phagocyte oxidase components, the 91-kd glycoprotein (gp91[phox]) and the 22-kd protein (p22[phox]), interact with the cytoplasmic components, the 47-kd protein (p47[phox]) and the 67-kd protein (p67[phox]). Glucose-6-phosphate dehydrogenase (G6PD) converts glucose-6-phosphate to 6-phosphogluconolactone, generating NADPH and a hydrogen ion from NADP+. NADPH oxidase catalyzes the monovalent reduction of O_2 to superoxide anion (O_2^-), with the subsequent conversion to hydrogen peroxide (H_2O_2) by superoxide dismutase. Neutrophil-derived myeloperoxidase (MPO) converts hydrogen peroxide to hypochlorous acid (HOCl− [bleach]), which is then converted to chlorine (Cl_2). The genes for the components of NADPH oxidase, their chromosomal locations, and the frequency of mutations as a cause of chronic granulomatous disease are indicated in the box.

autosomal mutations.[70] In the X-linked form, the onset is earlier, obstructive granulomas and infections are more frequent, and the mortality rate is higher. The reason for these differences is unknown. Interestingly, female carriers of X-linked chronic granulomatous disease, who have pronounced lyonization (inactivation) of the X chromosome, have levels of NADPH oxidase activity that are only 10 percent of normal levels, and this feature protects them from infections with catalase-positive organisms and the infectious sequelae of chronic granulomatous disease[77,78]; occasional patients with extreme lyonization who have
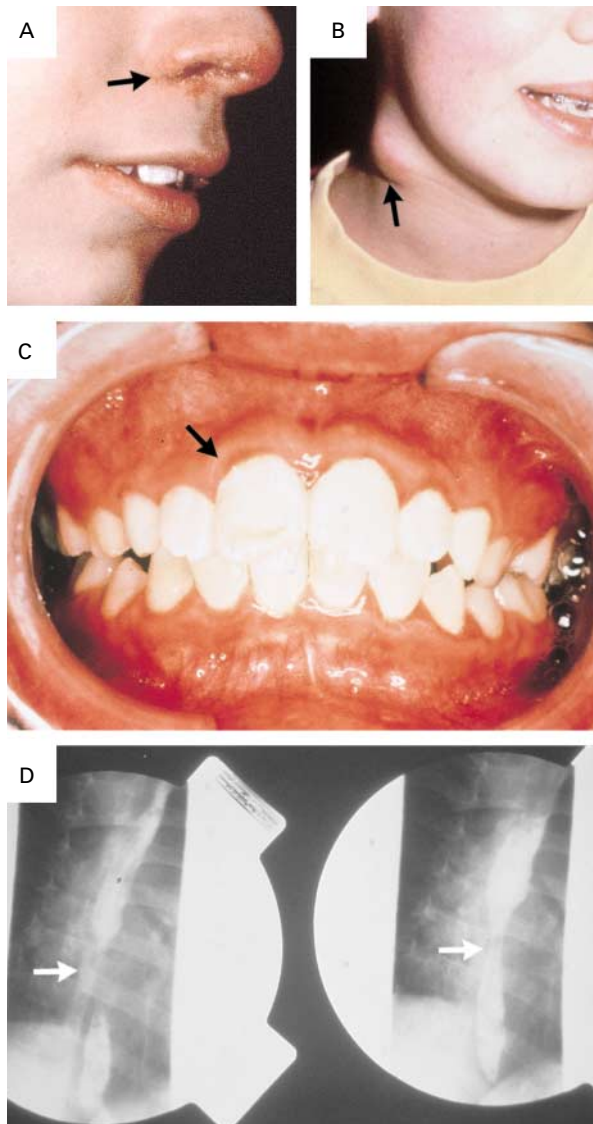
Figure 4. Clinical Features of Chronic Granulomatous Disease. Panel A shows painful inflammation of the nares. Panel B shows a large granuloma in the neck (arrow). Panel C shows severe gingivitis (arrow). On the left-hand side of Panel D, a barium swallow shows an esophageal stricture (arrow) caused by a granuloma. The granuloma resolved after treatment with intravenous methylprednisolone at a dose of 1 mg per kilogram of body weight every 12 hours for two weeks (right-hand side of Panel D). Panel D reprinted from Chin et al.[72] with the permission of the publisher.

fewer than 5 percent normal cells have clinically evident chronic granulomatous disease.[79]

In patients with chronic granulomatous disease, prophylactic treatment with trimethoprim–sulfamethoxazole (one single-strength tablet per day) reduces the frequency of life-threatening bacterial infections

from about once a year to once every four years without increasing the frequency of fungal infections.[80] Other treatments have also reduced infection rates. Treatment with interferon gamma reduces the incidence of opportunistic bacterial and fungal infections in patients with chronic granulomatous disease by over 70 percent.[81] Worldwide experience with allogeneic stem-cell transplantation for this disorder has been limited because of the high rates of morbidity and mortality associated with this procedure.[82] New approaches to stem-cell transplantation for chronic granulomatous disease have been reported recently using low-intensity marrow conditioning and T-cell depletion of the allograft. In a preliminary report on 10 patients, this approach was successful in 8 while reducing transplantation-related toxicity.[83]

Early clinical experience with gene therapy indicates that the transferred gene is detectable for six months.[84] Long-term success will most likely depend on finding the optimal combination of a gene-transfer vector and myeloablative conditioning.

DEFECTS IN THE FORMATION AND FUNCTION OF NEUTROPHIL GRANULES

Several genetic disorders of innate immunity stem from defects in the formation or function of neutrophil granules.

Myeloperoxidase Deficiency

Deficiency of myeloperoxidase is the most common inherited disorder of neutrophils. About half of those affected have a complete deficiency of myeloperoxidase; the rest have structural or functional defects in the enzyme.[85] Myeloperoxidase, the principal component of azurophilic (primary) granules, catalyzes the formation of hypochlorous acid (bleach) from hydrogen peroxide and chloride ion; hypochlorous acid is then converted to chlorine (Fig. 3).[86] Despite the ability of hypochlorous acid to kill microorganisms in vitro, a deficiency of myeloperoxidase is not generally associated with disease.[87,88] An important exception is patients with diabetes mellitus and myeloperoxidase deficiency, who are susceptible to disseminated candidiasis.[88] The mutations in the gene encoding myeloperoxidase are heterogeneous and can result in either transcriptional or post-transcriptional defects (Table 1).[89]

The Chédiak–Higashi Syndrome

The Chédiak–Higashi syndrome is an autosomal recessive disorder of all lysosomal granule-containing cells with clinical features involving the hematologic and neurologic systems.[90,91] Case reports by Chédiak[92] and Higashi[93] were published in the early 1950s, but the first cases were described in 1943.[91] In 1955, Sato recognized the similarities between Higashi's patients and Chédiak's patients and coined the term the "Chédiak–Higashi syndrome."[94] The
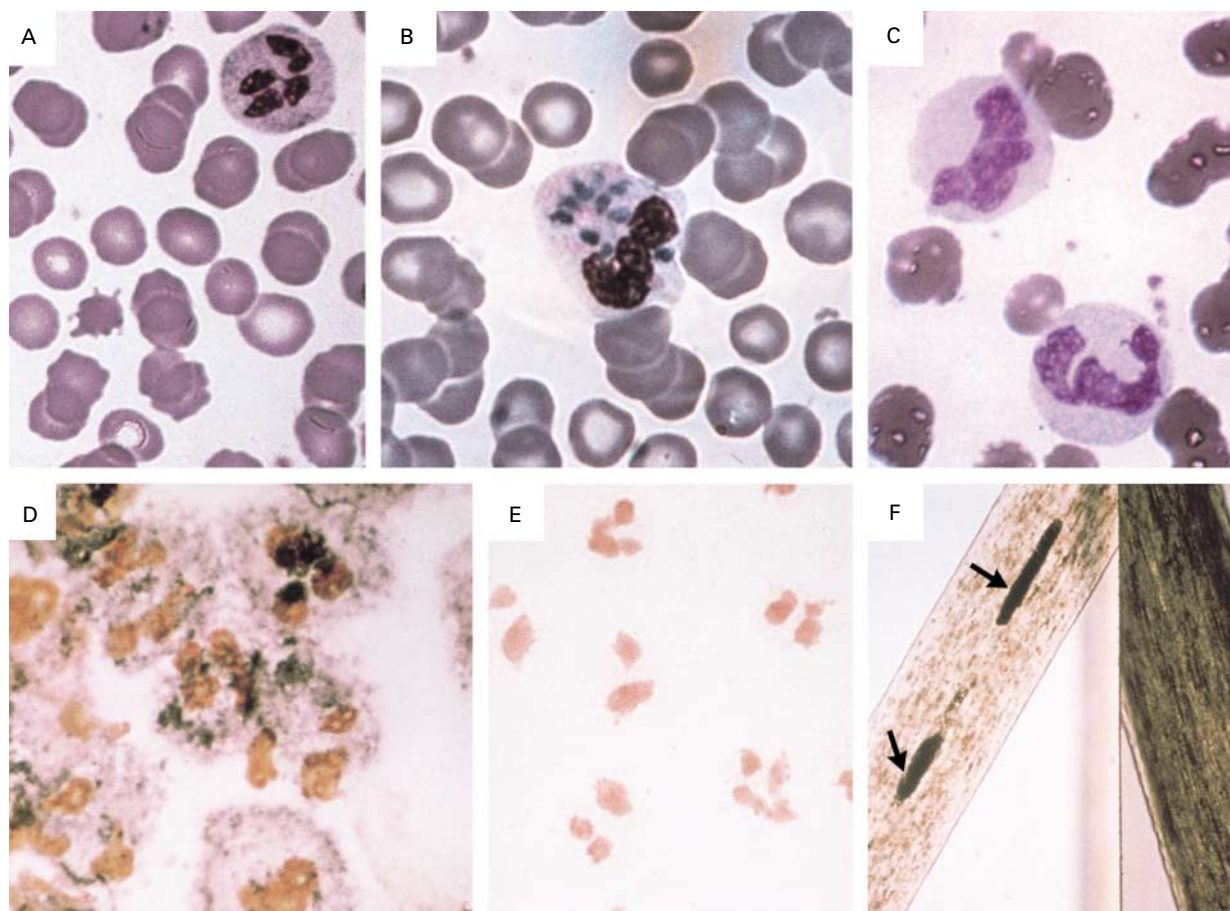
Figure 5. Diagnosis of Phagocytic Defect on the Basis of Light-Microscopical Findings.
Panel A shows a peripheral-blood smear from a normal subject (Wright–Giemsa, ×960). Panel B shows a peripheral-blood smear from a patient with the Chédiak–Higashi syndrome (Wright–Giemsa, ×960) in which there are large perinuclear granules. Panel C shows a peripheral-blood smear from a patient with neutrophil-specific granule deficiency in which the cytoplasm is pale (hyaline), no neutrophil-specific granules are present, and nuclei are notched and hyposegmented (Wright–Giemsa, ×960). Panel D shows the results of the nitroblue tetrazolium test in normal neutrophils: phagocytosis results in dark-blue staining of the cytoplasm (×960). Panel E shows the results of the nitroblue tetrazolium test in neutrophils from a patient with chronic granulomatous disease: there is no phagocytosis and thus no dark-blue cytoplasmic staining (×960). The left-hand side of Panel F shows a hair from a patient with the Chédiak–Higashi syndrome in which giant granules (arrows) are present, and the right-hand side of Panel F shows a hair from a normal subject (×450).

clinical features of the Chédiak–Higashi syndrome include recurrent bacterial infections, especially of *S. aureus* and beta-hemolytic streptococcus; peripheral nerve defects (nystagmus and neuropathy); mild mental retardation; partial ocular and cutaneous albinism; platelet dysfunction with easy bruising; and severe periodontal disease.[90,95] Patients have a mild neutropenia and normal immunoglobulin levels.[90] All cells containing lysosomes have giant granules (Fig. 5B). In neutrophils, the large granules result from the abnormal fusion of primary (azurophilic) granules with secondary (specific) granules,[96,97] and the fusion of the giant granules with phagosomes is delayed,

contributing to the impaired immunity.[90,98] Hair also has giant inclusions (Fig. 5F).

The mutated gene in the Chédiak–Higashi syndrome, *LYST*, encodes a cytoplasmic protein involved in vacuolar formation, function, and transport of proteins.[99,100] The neutrophils of patients with the Chédiak–Higashi syndrome fail to orient themselves correctly during chemotaxis as a result of a defect in the assembly of microtubules.[101,102] The neutrophils also lack the granule proteins elastase and cathepsin G.[103] The response to infection is a blunted neutrophilia and delayed diapedesis.[90]

In approximately 85 percent of patients with the

Chédiak–Higashi syndrome, the disease culminates in an often fatal infiltration of tissue by nonmalignant CD8+ T cells and macrophages, which requires therapy with lympholytic agents.[90] In patients who live until their late 20s, a striking peripheral neuropathy develops, which may be related to abnormal axonal transport as a result of the defect in microtubules. These patients become wheelchair-bound and usually die of infection in their early 30s.

Neutrophil-Specific Granule Deficiency

Neutrophil-specific granule deficiency is a rare but important disorder characterized by recurrent, severe infections with *S. aureus, S. epidermidis,* and enteric bacteria, primarily of the skin and lungs. The neutrophils of affected patients lack specific (secondary) granules, the granules that have an important role in inflammation.[104-109] Neutrophils with a deficiency of specific granules do not migrate normally, and they have atypical nuclear morphology (Fig. 5C).[105] In addition, neutrophils lack the primary-granule defensins,[103] and a deficiency of eosinophil-specific granules has been described.[110]

Mice with an inactivated gene for the CCAAT/ enhancer binding protein (*C/EBPε*) have a phenotype that is similar to that of patients with neutrophil-specific granule deficiency.[111-113] This protein is a member of the basic zipper family of transcription factors and is expressed nearly exclusively in myeloid-lineage cells.[114]

CONCLUSIONS

Congenital phagocytic defects must be included in the differential diagnosis of recurrent bacterial or fungal infections in a child or adult. The diagnosis is usually made during the first year of life, but leukocyte adhesion deficiency and chronic granulomatous disease may not be diagnosed until adulthood. The causative agents are often common commensal organisms of low pathogenicity, and certain microorganisms are associated with specific phagocytic defects. Infections with catalase-positive microorganisms are characteristic of chronic granulomatous disease, whereas infections with mycobacteria and other intracellular pathogens are typical of defects of the interferon-γ–interleukin-12 axis. Severe candidiasis in patients with diabetes suggests a deficiency of myeloperoxidase.

In patients who are thought to have defects in phagocytes, examination of the peripheral-blood phagocytes is essential, and characteristic abnormalities can often be identified with the use of a few simple stains. Supportive treatment of infections must be aggressive and involve broad-spectrum antibiotics and surgical drainage if necessary. Prophylactic treatment with interferon gamma is important in reducing the risk of infections in patients with chronic granulomatous disease and in some patients with defects of

the interferon-γ–interleukin-12 axis. Treatment with granulocyte colony-stimulating factor is a promising approach for some patients with well-defined phagocytic defects. Since the clinical manifestations of infection are often blunted as a result of impaired inflammation, diagnosis of phagocytic deficiencies and early intervention to treat infectious complications can be lifesaving.

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