Disorders of Neutrophil Function

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SUMMARY. Major bacterial infections are most commonly associated with agranulocytosis or an abnormality of immunoglobulins or complement. Occasionally, repeated infections cannot be attributed to these relatively common causes. In such cases, a quantitative abnormality in neutrophil function should be sought.

Complete evaluation of neutrophil function, including: chemotaxis, adhesion, aggregation, phagocytosis, granule content and degranulation, respiratory burst activity and bacterial killing is expensive and requires the services of a specialized laboratory. However, preliminary screening of a patient with a predisposition towards infection can be carried out using simple and inexpensive methods. These include examination of blood films, chemotaxis assay, NBT test and peroxidase staining. For final diagnosis and determination of genetic transmission and treatment, specific tests are indicated. Investigation of neutrophil functions may be useful for the diagnosis of congenital and acquired neutrophil disorders. These assays may also be useful in research, diagnosis and follow up of non-infectious diseases with active inflammatory component.

Neutrophils and mononuclear phagocytes are essential components of the host defense system, as can be inferred from patients whose neutrophils are greatly reduced in number or defective in function. Neutrophils originate in pluripotent stem cells that reside in the bone marrow. Colony-stimulating factors (multi-CSF, granulocyte-CSF, granulocyte-macrophage CSF) influence the stem cells to give rise to a committed population of neutrophils, which mature within 8–10 days. The neutrophil is packed with granules whose contents are essential to the killing and degradation of microorganisms. Azurophilic, or primary, granules contain proteases, hydrolytic enzymes, defensins and myeloperoxidase. Specific, or secondary, granules include enzymes such as collagenase, lysozyme, apolactoferrin and a C5 splitting enzyme. The encounter with microorganisms or other external stimuli triggers a cascade of events affecting neutrophil adhesion, migration and bactericidal functions.

The principal clinical manifestation of severe neutrophil malfunction is repeated bacterial infections. These are most commonly associated with pronounced neutropenia (<0.5 x 10^9/L) or abnormalities in either immunoglobulins or complement. In rare cases, when these are intact, a qualitative abnormality in neutrophil function should be sought as the cause for repeated infections.

Screening for abnormalities of neutrophil function entails the following tests:

1. Examination of the blood film. This can disclose large malformed granules in Chediak-Higashi disease, or bilobed nuclei with a distorted nuclear membrane in neutrophil-specific granule deficiency. Other morphological aberrations are Pelger Huet anomaly, seen in various stem cell...
disorders, Dohle-body-like inclusion bodies in May Hegglin anomaly, and hypersegmentation in megaloblastic anemia.

2. **Rebuck skin window.** This measures migration of neutrophils on to a glass coverslip applied to a superficial abrasion. Alternatively, neutrophil chemotaxis can be measured in vitro using the Boyden chamber and neutrophil adhesion can be determined in vitro by the cells' adherence to nylon wool. Migration is impaired in neutrophil-specific granule deficiency as well as in leukocyte adhesion deficiency (LAD) and in Chediak–Higashi syndrome.

3. **Respiratory burst.** This is evaluated by the Nitroblue tetrazolium (NBT) reduction test. In this assay, cells are activated in the presence of the dye NBT which forms a dark precipitate in cells producing superoxide (O$_2^-$). Respiratory burst activity can be measured directly as oxygen consumption, O$_2^-$ production (using cytochrome C reduction) or hydrogen peroxide production, and indirectly by measuring neutrophil chemiluminescence.

4. **Special stains** for myeloperoxidase and alkaline phosphatase are used to detect deficiencies in these enzymes.

Additional tests include: **Degranulation** – release of certain enzymes which can be directly measured and used to differentiate between a defect in the primary or secondary granules, and **phagocytosis and killing of bacteria and fungi**. These tests are carried out mainly for research purposes and are not used for routine screening of patients with a tendency toward pyogenic or fungal infections.

In this review, we shall discuss benign congenital neutrophil disorders in which a correlation between the in vitro abnormality and the clinical manifestations have been established (Table 1). Neutrophil defects associated with other systemic diseases will also be briefly addressed.

### Chronic Granulomatous Disease of Childhood (CGD)

CGD refers to a group of inherited X-linked and autosomal recessive disorders in which phagocytes (neutrophils, eosinophils and monocytes) are unable to express respiratory burst. Defects in the various components of the complex enzyme system nicotinamide–adenine dinucleotide phosphate (NADPH) oxidase result in CGD.

The enzyme responsible for the reduction of O$_2$ to O$_2^-$ includes various cellular components present in the cytosol and in the cell membrane. This enzyme system contains a flavoprotein and the unique cytochrome b$_558$. Cytochrome b$_558$ consists of a large 91 kd subunit (gp91phox) and a small 22 kd protein subunit (p22phox). Two cytosolic components (p47phox and p67phox) form a complex with cytochrome b$_558$ and interact with a cytosolic GTP binding protein, rac2. As shown in Table 2, the most common form of CGD, found in about two thirds of patients, is due to an abnormality of the membrane-associated heavy chain of cytochrome b$_558$ encoded by the X chromosome. Deficiency of the cytosolic protein product, p47-phox encoded by a gene in chromosome 7 is present in a quarter of the patients. Deficiency of the light chain of cytochrome b$_558$, p22-phox, linked to chromosome 16, and a lack of the larger cytosolic protein, p67-phox, encoded by chromosome 1, each account for ~5% of the patients suffering from CGD.

### Classification of chronic granulomatous disease

<table>
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<th>Affected component</th>
<th>Gene locus</th>
<th>Inheritance</th>
<th>Location of defect</th>
<th>Frequency (% of cases)</th>
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<td>p67-phox</td>
<td>1q25</td>
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</table>

Clinical Manifestations

Patients suffer from recurrent infections by catalase positive microorganisms (Staphylococci, Enterobacteria, and fungi). Since the bacteria are ingested but not killed and digested, patients tend to develop severe chronic granulomas. The most common infections involve the lungs, skin, gastrointestinal tract and lymph nodes draining these tissues or organs.

Chronic granuloma formation in these patients may lead to several non-infectious complications, such as hepatosplenomegaly, lymphadenopathy, hypergammaglobulinemia, chronic diarrhea and granulomatous obstruction of certain organs. Patients become chronically ill and their ability to withstand frequent infections deteriorates. Death usually results from fungal infections, mainly caused by *Aspergillus* species.

### Diagnosis

Diagnosis is established by the NBT test or other tests proving lack of phagocyte O$_2^-$ production. Characterization of the mutations in CGD makes it possible to provide earlier and accurate prenatal diagnosis of CGD using fetal DNA from chorionic villus samples.
villi or amniocytes. This is carried out in a limited number of laboratories around the world. In the absence of molecular genetic facilities, prenatal diagnosis can be established using the NBT test on small amounts of fetal blood obtained by percutaneous umbilical sampling.

Treatment

Prophylactic antibiotic therapy, especially with trimethoprim-sulfamethoxazole reduces the incidence of life-threatening infections and is routinely provided to patients with CGD. This has resulted in improved life expectancy and quality but also in the emergence of severe fungal infections. A study introducing prophylactic anti-fungal therapy to these patients is currently being carried out (Malech HL, personal communication). Early vigorous antibiotic treatment of infections by resistant bacteria or fungi is essential, as is surgical drainage of granulomas when indicated. In some life-threatening infections, leukocyte transfusion may be useful. Granulomas causing obstruction in vital organs can regress upon treatment with hydrocortisone, presumably by affecting the production of cytokines such as Interleukin-1, Gamma Interferon (γIFN) and tumor necrosis factor (TNF). This therapeutic modality, however, is controversial and should be administered with caution.

Recently, it was reported that γIFN reduces the incidence and severity of infections in patients with CGD. The mechanism of action was thought to involve stimulation of the macrophage oxidative pathway, especially the heavy b cytochrome chain of the NADPH oxidase. However, in 63 patients receiving γIFN in a Phase III trial, none showed an increase in neutrophil O₂⁻ production, despite obvious clinical improvement. This suggests that the cytokine does not augment the host defense system by reversing the respiratory burst defect, but by a mechanism that remains to be established. γIFN is indicated as a therapeutic agent in CGD patients along with antibiotics, both on a continuous basis. Anti-fungal maintenance therapy is currently under investigation. Bone marrow transplantation is rarely performed because of improvement in the quality of life and survival of the patients, and since the results of transplantation have usually proved disappointing.

Neutrophil Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

NADPH, the primary substrate for the respiratory burst oxidase, is generated by the first two reactions of the hexose monophosphate shunt pathway, in which the first enzyme, G6PD, is deficient in certain populations. Despite the high frequency of this enzyme deficiency in erythrocytes, severe G6PD deficiency in neutrophils, causing a CGD-like clinical picture, is extremely rare. One of the reasons it does not occur more frequently is the short life span of the neutrophil as opposed to that of the red blood cell, allowing the enzyme to be used up before the cell dies. Actually, a CGD-like syndrome due to neutrophil G6PD deficiency has only been described in patients with extremely low levels of the enzyme who also express nonspherocytic hemolytic anemia.

This unique clinical association, coupled with extremely low neutrophil and erythrocyte G6PD levels is used to distinguish this disease from CGD. The treatment for neutrophil G6PD deficiency is the same as for CGD, except that the effect of γIFN is questionable and supportive therapy for chronic hemolysis is required.

Myeloperoxidase (MPO) Deficiency

Myeloperoxidase (MPO) deficiency, the most common inherited disorder of neutrophil function, is transmitted as an autosomal recessive trait and affects approximately 1:2000 persons. Fifty percent of such patients display a total lack of myeloperoxidase, others have partial deficiencies. The gene encoding for MPO is located on chromosome 17 at q22–q23, near the breakpoint for the 15;17 translocation of acute promyelocytic leukemia. The cloning of myeloperoxidase cDNA and the demonstration of mRNA transcripts in bone marrow suggest that, in some of the patients, MPO deficiency results from impaired post-translational processing of abnormal precursors. Multiple restriction fragment length polymorphisms (RFLPs) have been identified in patients with both complete and partial MPO deficiency, suggesting the heterogeneous nature of the disease.

Clinical Manifestation

Most of the patients do not have serious bacterial infections since the respiratory burst remains essentially intact. Nevertheless, Candida infections are frequent, particularly when other illness, such as diabetes mellitus, coexists.

Diagnosis

MPO-deficient neutrophils display normal chemotaxis, phagocytosis and degranulation. The respiratory burst is mildly prolonged and hydrogen peroxide production is mildly augmented. Bacterial killing is delayed, but eventually reaches completion. Candidacidal activity is usually defective. Diagnosis is made by peroxidase staining of blood films which demonstrates deficient MPO in neutrophils and monocytes. Eosinophils stain normally since they contain a different peroxidase.

Treatment

Since most of the patients do not suffer from life-threatening infections, maintenance antibiotic ther-
apy is not recommended. Caution is required in diabetic patients, who suffer from a high tendency toward pyogenic infections, and concomitant MPO deficiency makes them prone to fungal infections.

Leukocyte Adhesion Deficiency

Leukocyte adhesion deficiency (LAD) is a rare disorder of leukocyte adhesion and chemotaxis that results in severe bacterial infections. The molecular basis for LAD involves three integrins (adhesion molecules) all sharing identical β2 subunit (CD18) and distinct α subunit (CD11a,b,c). Patients express either structurally abnormal, or reduced amounts of the β subunit, resulting in a deficiency of all three integrins. Various mutations, leading to various degrees of severity in clinical manifestation, have been identified in the gene encoding for the β2 subunit.

Reduced, or lack of, expression of all three β2 integrins in leukocytes of patients with LAD results in two major functional defects. One is the failure of the phagocyte to adhere to, and to migrate through, the endothelial lining. The other is the inability of the leukocyte to bind C3bi opsonized microorganisms. Since CD11b/CD18 is the C3bi receptor (C3R) for the neutrophil, all phagocytic functions dependent on this opsonin receptor (i.e. C3bi mediated phagocytosis, enzyme release and O2- production) are severely affected. This combined dysfunction leads to a severe clinical picture in most cases, although patients with a moderate tendency towards infection were described.

Recently, Etzioni et al. described two unrelated children with a defect in leukocyte adhesion due to the absence of the Sialyl-Lewis X ligand of the neutrophil E-selectin and P-selectin counter receptors. Since these patients have normal levels of CD18 integrin expression, the syndrome was designated leukocyte adhesion deficiency type II (LAD II).

Clinical Manifestations

The first indication of this disease may be delayed separation of the umbilical cord. Recurrent infections, especially by pseudomonas species, are common, as are impaired formation of pus and high leukocyte counts. The clinical presentation of LAD I is, however, heterogeneous and correlated with the severity of the β2 integrin deficiency. In patients suffering from LAD II, mental retardation, short stature, a distinct facial appearance and Bombay (hh) blood phenotype were observed, in addition to the tendency towards pyogenic infections, perhaps reflecting a profound defect in fucose metabolism.

Diagnosis

Decreased neutrophil chemotaxis and adhesion raise the possibility of LAD. Deficient CD18 (or CD11b) upon flow cytometry with monoclonal anti CD18 (or anti CD11b) is used to establish a diagnosis of LAD I. Deficient Sialyl-Lewis X is indicative of LAD II.

Treatment

Prolonged therapy with antibiotics is usually necessary to control infections, and aggressive use of parenteral antibiotics is crucial during infection with resistant microorganism. Severely afflicted patients (CD11b < 0.3% normal) with an HLA-matched donor may be eligible for allogeneic bone marrow transplantation.

Localized Juvenile Periodontitis

In this familial disorder, serious gingival inflammation develops in childhood or adolescence resulting in severe alveolar bone loss of the first molars and incisors. Among the organisms infesting the gums is the bacterium Capnocytophaga which secretes an inhibitor of neutrophil chemotaxis. Most of the patients are not predisposed to serious systemic infections and treatment is therefore local.

The generalized form of prepubertal periodontitis is now identified as the oral manifestation of the leukocyte adhesion deficiency syndrome. The pathogenesis of this disease at the molecular level is known, though its localization to the oral cavity remains obscure. Localized prepubertal periodontitis is a disease defined by clinical criteria whose molecular basis remains to be established.

Hyperimmunoglobulin E Syndrome (Job Syndrome)

Job syndrome is characterized by cold abscesses and chronic dermatitis beginning in early childhood, and markedly increased IgE (at least ten times the normal value). The response to inflammation is impaired in these patients, but the etiology has not been elucidated. Neutrophil and monocyte chemotaxis is impaired to a variable extent. A chemotactic inhibitory factor produced in vitro by the phagocytic cells of such patients has been described, and mitogen- and antigen-induced lymphocyte transformation in response to tetanus and Candida antigens is impaired. Excessive production of IgE against Staphylococcus aureus, occurring at the expense of protective anti-staphylococcal IgA could contribute to the recurrent sinopulmonary infections by this pathogen. Increased IgE and abnormal neutrophil chemotaxis might be related to decreased production of γIFN and TNF. Evidence favoring this assumption relies on studies demonstrating decreased γIFN production by T lymphocytes of patients with Job syndrome when exposed in vitro to mitogenic stimuli, and on the finding that IgE production by B lymphocytes can be reduced by addition of this cytokine.
Clinical Manifestations

Patients suffer from recurrent cutaneous and sinopulmonary bacterial infections, mainly involving *Staphylococcus aureus* and *Hemophilus influenzae*. Cold staphylococcal abscesses lacking the typical features of acute inflammation are present, as well as eczematous rashes. Characteristic facies with hypertelorism, prominent jaw and osteoporosis are present.26

Diagnosis

Job syndrome is suspected when patients present with recurrent bacterial sinopulmonary and skin infections from birth or early childhood. A characteristic finding is a high IgE level, at least 10 times greater than the upper normal limit, directed mainly against *S. aureus*. Impaired neutrophil chemotaxis establishes the diagnosis.

Treatment

The mainstay of the therapy is treatment with antibiotics for infections, topical steroids and emollient creams for the eczematous rashes, and surgical incision and drainage of the abscesses. Treatment with prophylactic antibiotics is controversial. Administration of γIFN is now being investigated in view of the fact that this cytokine has been documented to improve the chemotaxis of the patients' neutrophils in vitro.29

Congenital Absence of Specific Granules (SGD)

Specific (secondary) granules contain leukocyte adhesion molecules, various enzymes important for modulation of the inflammatory cascade (i.e. lactoferrin, histaminase) and components of the oxidase system.30 Specific granule-deficient neutrophils lack the contents of the secondary granules and are also devoid of defensins present in the primary granules, suggesting defective regulation of the synthesis of various lysosomal proteins.31 This results in decreased chemotaxis, impaired O2− production and low bactericidal activity.31 The precise molecular defect responsible for SGD has not been identified. However, the putative fault in the regulation of protein synthesis appears to be confined to the myeloid series since cells other than myeloid precursors show normal expression of lactoferrin, despite the severe deficiency in neutrophils.30

Clinical Manifestations

The two fundamental defects in the neutrophils of SGD patients, namely the absence of the intracellular pool of leukocyte adhesion molecules, resulting in a severe chemotactic defect, and deficiency in microbicidal molecules (lactoferrin, defensins, components of the oxidase system) result in recurrent bacterial and fungal infections, involving mainly the skin and lungs.30,31

Diagnosis

Diagnosis of SGD can be readily made by light microscopy. Though normal in quantity, the Wright-stained neutrophils contain normal numbers of primary granules but lack secondary granules. Bilobed nuclei are sometimes present. Diagnosis can be established by electron microscopy or by the direct demonstration of a severe deficiency of lactoferrin, B12 binding proteins or defensins. In addition, impaired chemotaxis and respiratory burst are observed.30,31

Treatment

Treatment is similar to that for other neutrophil disorders, namely, prophylactic antibiotics, vigorous parenteral treatment of acute infections and surgical drainage when indicated. Among the few families described, the patients reached adulthood, provided medical management was prompt and aggressive.

Chediak–Higashi Syndrome

Chediak Higashi syndrome is a rare autosomal disease defined by the presence of giant lysosomal granules in all blood cells, including phagocytes, lymphocytes and platelets.32 The giant granules are formed by the fusion of azurophilic and secondary granules. Cathepsin G and elastase concentrations are reduced. Abnormal cyclic nucleotide metabolism, aberrant microtubule assembly and increased tyrosination of the tubulin α chain probably account for the observed impairment in migration and orientation of the neutrophils.33 Oxygen consumption and hydrogen peroxide production are enhanced.34 Lymphocytes from patients with Chediak–Higashi have decreased NK activity and antibody-dependent cell mediated cytotoxicity is impaired.34 The etiology for this defect has not been elucidated.

Clinical Manifestations

The main symptoms result from malfunction of lysosome containing cells and include: partial albinism due to melanocyte malfunction; bleeding disorder secondary to a platelet defect resembling storage pool disease and causing prolonged bleeding time, but usually only a minor bleeding tendency; low resistance to infection due to phagocyte abnormalities resulting in recurrent bacterial infections (sinusitis, pneumonia) and deep-seated abscesses.32 Infections are life-threatening and many patients die in the first decade of life. Patients who reach the second decade enter an accelerated phase characterized by pancytopenia due to infiltration of the bone marrow, lymph
nodes and liver with small polyclonal lymphocytes and histiocytes. Though histopathologically benign, the clinical course resembles the aggressive virus-associated hemophagocytic syndrome that may be precipitated by uncontrolled EBV infection. Death usually occurs several months after the development of pancytopenia, unless bone marrow transplantation is performed.

Diagnosis

Diagnosis is established by the presence of giant peroxidase-positive lysosomal granules in all blood cells. Impaired neutrophil chemotaxis and degranulation have also been demonstrated.

Treatment

Early treatment of infections and prophylactic antibiotic therapy are vital to management of the patients in the early phase of the disease. Prompt treatment with ascorbate, as described by Boxer et al., is controversial. In the accelerated phase, both chemotherapy and splenectomy have proved futile. Bone marrow transplantation is a therapeutic option. However, the time for transplantation remains to be established, since at the advanced stage, when the patient is chronically ill, the success rate is low.

Secondary Neutrophil Function Defects

Various neutrophil defects have been described in hematological and non-hematological disorders. These functional abnormalities are usually partial and therefore a tendency toward infection is uncommon and not severe. The issue was recently reviewed and will be addressed in brief.

Hematological Disorders

Acute Non-Lymphocytic Leukemia (ANLL)

Patients with ANLL sometimes suffer from infections despite a normal neutrophil count and it has been postulated that the latter are functionally defective. Indeed, deficiency in one or more of the neutrophil enzymes has been occasionally described.

Myeloproliferative Disorders

Patients with chronic myeloid leukemia (CML) usually do not suffer from recurrent infections during the chronic phase of the disease. Nevertheless, functional abnormalities such as low alkaline phos phatase activity, abnormal adhesiveness and reduced migration are common. Clinically significant infections are not usual in polycythemia vera, essential thrombocytosis and myelofibrosis with agnogenic myeloid metaplasia.

Myelodysplastic Syndrome (MDS)

Even in the presence of normal neutrophil counts, there is a predisposition to infections prior to transformation to acute leukemia. According to several reports, this may be attributed to secondary qualitative abnormalities in neutrophil adhesion, migration, phagocytosis and microbicidal abilities.

Lymphoproliferative Disorders

In these disorders, the tendency towards pyogenic infections is secondary to deficient humoral factors, such as immunoglobulins in multiple myeloma and chronic lymphocytic leukemia. Inhibitory substances in the serum of certain patients were described and reduced granulocyte counts may result from circulating antigranulocyte antibodies. Granulocyte dysfunction in lymphoproliferative disorders is exceptional.

In summary, direct neutrophil dysfunction has been proven in certain non-lymphatic hematologic disorders, while serum immunodeficiencies and inhibitory substances have been described in lymphatic malignancies. However, these studies must be critically evaluated since not all have been performed under optimal conditions, namely in afebrile and untreated patients (see next paragraph). Despite this reservation, patients with lymphoid and non-lymphoid hematologic malignancies (mainly MDS) should be closely monitored for infectious episodes, even when sufficient numbers of seemingly mature neutrophils are present. Evaluation of neutrophil function (in MDS) and studies of serum immunoglobulins (in lymphoid malignancies) may be useful in screening for those patients who are more likely to develop infectious complications.

Non-hematological Disorders

The most important non-hematological disorder associated with a tendency towards pyogenic infections is diabetes mellitus. In these patients, especially those with uncontrolled hyperglycemia, a chemotactic and adhesion defects were described. The correlation between this laboratory finding and infections has not been proved but patients with concomitant MPO deficiency are prone to fungal infections. Depressed neutrophil activity, mainly chemotaxis, has also been reported in cirrhosis of the liver, severe burns and in premature neonates. This can partially account for the decreased resistance to bacterial infections characteristic of these disorders. In addition, it is known that during a septic episode or a protracted infectious course, neutrophil function may be altered. Splenectomized patients tend to be more susceptible to infections, due to a defect in opsonization along with impairment of granulocyte
chemotactic and phagocytic functions. Cytotoxic agents, corticosteroids and ionizing radiation can also modify neutrophil activity. Again, neutrophils, corticosteroids and ionizing radiation can chemotactic and phagocytic functions. There are reports of increased neutrophil adhesiveness during severe bacteremia and hemodialysis, acting to increase pulmonary symptoms due to trapped neutrophils. In familial Mediterranean fever, psoriasis vulgaris, Behçet's syndrome and Sweet's syndrome, increased neutrophil chemotaxis may be helpful in the diagnosis and followup, particularly when evaluating the response to antiinflammatory agents such as colchicine.

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References


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