HOST DEFENSES AGAINST INFECTION: IMPORTANCE OF PHAGOCYTIC MECHANISMS FROM THE STUDY OF GENETIC DISORDERS OF LEUKOCYTE FUNCTION

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WITH the complement system, phagocytic cells play a crucial role in early defense against bacterial and fungal infections. The past two decades have witnessed a tremendous increase in understanding of the mechanisms whereby these two systems deal with invading organisms. Much of this information has been derived from studies of patients with various genetic deletions of host defense. This brief review will center on phagocytic cells and on how genetic disorders of their functions have helped to define their normal operation. More detailed reviews of the complement [1-3] and phagocytic cell systems [4-6] have recently appeared.

RECOGNITION OF DISORDERED PHAGOCYTIC DEFENSES

Patients with disorders of the number of functions of phagocytic cells can be distinguished by the types of infections that they develop. Subjects with abnormalities in antibody formation or in the complement system are infected by encapsulated bacteria.[7] In contrast, organisms causing infections in patients with neutropenia or phagocytic cell dysfunction are usually bacteria of relatively low virulence or opportunistic fungi (Table I). Encapsulated bacteria are obligate extracellular parasites and are rapidly killed when phagocytized. Antibody or the third component of complement or both modify the surface characteristics of these organisms. This results in their prompt phagocytosis, mediated in part by their interaction with cellular receptors for the Fc portion of IgG and C3b.[8] Both Gram negative and Gram positive organisms may activate the complement

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TABLE I. ROLE OF HOST DEFENSES AGAINST INFECTION

Defense	*Infectious complications when impaired*
1) Local (drainage, mucosal and skin barriers, etc.)	Bacteria > fungi > viruses and protozoans
2) Inflammatory (PMNs and complement: phagocytosis and killing)	Bacteria + fungi > viruses and protozoans
3) Antibodies (B-cells; antitoxins, neutralizing, opsonins, antiadherence)	Bacteria + protozoans > fungi and viruses
4) Cellular immunity (T-cells and macrophages' killing of intracellular organisms)	Intracellular bacteria, fungi, viruses, and protozoans

system through the alternate pathway or when IgM or IgG antibodies bind to their surface and lead to the fixation of the C1 complex (classical pathway). Besides promoting opsonization when C3b is bound to the bacterial cell surface, many Gram negative organisms are subject to lysis by the terminal membrane attack complex (C6-9) of the complement system.[1,2] Genetic deletions in any of these terminal components have resulted in an increased incidence of disseminated Neisserial infections.[2,7] This emphasizes the importance of lytic complement factors in addition to antibody in protection against these organisms.

Staphylococci frequently cause infections in patients with neutrophil disorders, and are readily opsonized by either antibody or complement.[5,9] Thus, a deletion in one or the other system is not likely to have major consequences for the phagocytosis of those organisms. On the other hand, staphylococci are more resistant to intracellular killing than encapsulated bacteria, in part related to their content of the enzyme catalase.[10] Catalase activity may be an important microbial virulence factor for some organisms by destroying H_2O_2 formed by phagocytic cells. The Gram negative rods and opportunistic fungi which also cause infections in many patients with neutrophil disorders are catalase-positive, but some patients are also susceptibile to infection by catalase-negative species as well (see below).

The organisms that cause infection in patients with altered cellular immunity are often quite resistant to intracellular killing by neutrophils

and macrophages. The mechanisms by which these organisms resist killing are beginning to be elucidated.[11] Cellular immune mechanisms appear to be important in defense against mucosal and cutaneous infection with *Candida* species, but neutrophils play a key role in defense against visceral invasion by these organisms.[12] Thus, the pattern of the infections and the characteristics of the organisms isolated can provide important clues regarding the nature of altered host defense mechanisms.

Disorders of Phagocyte Adherence

Both polymorphonuclear leukocytes and monocytes spend a relatively brief time in the circulation.[4,13] Their functions are normally restricted to the extravascular space (polymorphonuclear leukocytes and tissue macrophages) or to intravascular sites in certain organs (e.g., splenic, hepatic, and lung macrophages).

Adherence to endothelial cells in postcapillary venules over sites of inflammation has been defined as an important initial stage in the inflammatory process.[4] Adherence may be reduced by a heat labile plasma factor which is induced when subjects ingest large amounts of alcohol or are given high doses of glucocorticoids or nonsteroidal anti-inflammatory agents or by such compounds as epinephrine which induce adenyl cyclase activity.[14,15] The leukocytosis induced by glucocorticosteroid administration as well as the failure of polymorphonuclear leukocytes to enter inflammatory sites in patients administered these compounds may in part reflect the reduced adhesiveness of these cells.

Recently, a very interesting patient with an X-linked defect in the formation of a granulocyte membrane glycoprotein ("Gp 110") has been described. His cells had markedly reduced adherence and spreading on glass or plastic surfaces, impaired random motility and chemotaxis, and impaired ingestion of particulate materials. The presumed function of this protein is to promote contact between the neutrophil membrane and other surfaces, i.e., "anchor" the cells to the surfaces. Because of this phenomenon, the term "anchor disease" has been coined for this disorder. Clinically, the affected boy has had a chronic polymorphonuclear leukocytosis and recurrent skin, ear and airway infections by a variety of bacteria.[16]

Disorders of Chemotaxis

Polymorphonuclear leukocytes and monocytes leave the circulation at sites of inflammation or infection by penetrating between endothelial cells in response to increasing gradients of chemotactic factors. Chemotactic

TABLE II. NATURE OF DEFECTS OF LEUKOCYTE FUNCTION

Function	*Defect*	*Clinical conditions*
1) Delivery		
a) Production and destruction	↓ number of mature cells	Neutropenias, leukemias
b) Adhesion	↓ adhesiveness at inflammatory site	Anti-inflammatory drugs, alcoholism, "Anchor" disease
c) Chemotaxis	↓ cellular responses	Chediak-Higashi Syndrome, "lazy leukocyte," actin deficiency, Kartagener's syndrome, serum inhibitor(s), Hyper IgE syndrome
	↓ production of chemotactic factors	Depletion of complement (C_3), serum inhibitors
2) Attachment and ingestion	Depressed phagocytosis	Cellular immaturity, hyperosmolarity, acidosis, actin deficiency, "Anchor" disease, GP_{150} disease
	↓ cellular function	
	↓ "humoral" (opsonizing) function	Antibody deficiency, complement (C_3) deficiency
3) Intracellular Killing	↓ oxygen-dependent mechanisms	Hypoxia, chronic granulomatous disease, G6PD deficiency, myeloperoxidase deficiency, Chediak-Higashi syndrome
	↓ non-oxygen dependent mechanisms	Chediak-Higashi syndrome, miscellaneous defects

factors can include cleavage products of the complement system (C3a, C5a), bacterial or fungal products or factors released from injured tissues.[17] Disordered chemotaxis can result from failure of chemotactic factors to be generated, failure of cells to respond, or disordered regulation of chemotaxis by normally inhibiting mechanisms.[18] Some examples of each of these types of disorders are shown in Table II and have been more completely reviewed elsewhere.[5,18]

Reduced chemotaxis has been described in several acquired disorders complicated by an increased incidence of infection, including diabetes mellitus[19] and rheumatoid arthritis.[20] The importance of this finding relative to other factors in these diseases which may reduce host defense needs to be more clearly defined.

Recent data indicate that impaired chemotaxis may be corrected in some patients with the lysosomal disorder, the Chediak-Higashi syndrome, by administration of ascorbate.[21] Ascorbate augments chemotaxis of normal cells *in vitro* but may not have similar effects *in vivo*.[18,22] Chemotaxis may be improved in the disorder known as "Job's syndrome" (hyper IgE, eosinophilia, and recurrent staphylococcal infections) by levamisole.[18,23] Levamisole appears to operate by suppressing production of a mononuclear cell factor, which inhibits polymorphonuclear chemotactic responses.[24] The general utility of this compound will have to be established.

Disorders of Microbial Killing

Chronic granulomatous disease. The importance of oxygen in the normal antimicrobial function of polymorphonuclear leukocytes[4-6] was revealed largely by the study of patients with genetic disorders of phagocyte function. Baldridge and Gerard first described the "extra respiration" of phagocytosis.[25] The studies of Cohn and Morse and Sbarra and Karnovsky indicated that most of the oxygen was not consumed by mitochondrial metabolism.[26,27] Iyer and coworkers observed that phagocytosis was associated with increased production of H_2O_2.[28] The relevance of these observations to the antimicrobial mechanisms of polymorphonuclear leukocytes was clarified in 1967 with the reports that boys with the disorder known as "fatal granulomatous disease of childhood" (later called "chronic granulomatous disease" (CGD)) had impaired intracellular killing of *S. aureus* and failure to consume oxygen or to produce H_2O_2 during phagocytosis.[29,30] At about the same time, Klebanoff and McRipley and Sbarra independently defined an antimicrobial system of polymorphonuclear leukocytes which involved the action of the azurophilic granule enzyme, myeloperoxidase, H_2O_2 and halide cofactors.[31,32] Thus, it seemed likely that the antimicrobial defect in chronic granulomatous disease was a consequence of impaired production of H_2O_2. These findings were later extended to demonstrate that polymorphonuclear leukocytes, monocytes, and eosinophils from patients with chronic granulomatous disease could not normally kill a variety of catalase positive bacteria and fungi.[33-35]

In metabolic studies it was shown that cells from patients with chronic granulomatous disease lacked not only a burst in oxygen consumption during phagocytosis, but hexose monophosphate shunt activity was also markedly impaired as was myeloperoxidase-mediated iodination of proteins and the formation and release of H_2O_2.[30,36,37] Further, phagocytizing

polymorphonuclear leukocytes from patients with chronic granulomatous disease lacked the ability to reduce the electron-accepting dye nitroblue tetrazolium.[38]

The interrelationships between these metabolic events were clarified when Babior and coworkers demonstrated that normal cells produced but polymorphonuclear leukocytes from patients with chronic granulomatous disease failed to make the one electron reduction product of oxygen, superoxide, during phagocytosis.[39,39a] Later work demonstrated that during phagocytosis the major product of the respiratory burst of polymorphonuclear leukocytes was O_2-, which underwent spontaneous dismutation to H_2O_2.[40] The H_2O_2 produced could be catabolized in a variety of ways including by catalase, glutathione peroxidase, and, in phagocytic vacuoles, by myeloperoxidase.[41] The catabolism of H_2O_2 by glutathione peroxidase could be associated with stimulation of the hexose monophosphate shunt[42] and utilization of H_2O_2 by myeloperoxidase to the halogenation of proteins.[31] The stimulated reduction of nitroblue tetrazyolium was shown to be an indirect method of measuring O_2- formation,[43] and provides the basis for simple tests for detecting patients with chronic granulomatous disease.[38,44]

Subsequent studies have shown that the metabolic defect in chronic granulomatous disease can be ascribed to a failure of a (presumably) plasma membrane bound oxidase to reduce molecular oxygen to O_2- during activation by phagocytosis or other forms of membrane stimulation.[39a,45] The major electron donor in this reaction is NADPH, and a cytochrome b may be involved as an intermediate in electron transfer to O_2.[46] Classic chronic granulomatous disease patients exhibit sex-linked inheritance and fail to activate this oxidase normally. Variant forms have been described in which substrate production is poor (e.g., severe G6PD deficiency) or oxidase affinity for NADPH is decreased.[4-6,47,48] Normal neutrophils may behave like those in chronic granulomatous disease when placed in an anaerobic environment.[49] Finally, local anaerobiasis has been suggested to contribute to the failure of organisms to be eliminated by polymorphonuclear leukocytes in some chronic infections such as osteomyelitis.[50]

Catalase-negative organisms such as pneumococci are killed normally by cells of patients with chronic granulomatous disease by suicidally supplying small amounts of H_2O_2 during their metabolism.[35] Similarly, the abnormal killing of catalase positive organisms by these cells can be corrected *in vitro* by provision of exogenous H_2O_2 as can some of the

metabolic abnormalities (hexose monophosphate shunt activity and protein iodination).[51,52] These findings indicate the importance of H_2O_2 to polymorphonuclear leukocyte antimicrobial activity and suggest means by which the defects in chronic granulomatous disease cells might be corrected. However, their translation to the treatment of these patients awaits further investigation.

Myeloperoxidase deficiency. Myeloperoxidase comprises 5% of the wet weight of a normal neutrophil and is localized to the azurophilic granules.[53] It is a hemoprotein also found in monocytes [54] and is structurally, antigenically, and biochemically distinct from eosinophil peroxidase.[55-57] Myeloperoxidase utilizes H_2O_2 as a substrate to catalyze the oxidation of halide ions to their hypohalite derivates.[6,49] These compounds are also strong oxidants that can halogenate proteins, cleave peptide bonds, decarboxylate amino acids, or react with H_2O_2 to form other strong oxidants such as singlet oxygen.[4,6,49]

Primarily through the work of Kelbanoff and coworkers, myeloperoxidase has been shown unequivocally to operate in normal polymorphonuclear antimicrobial actions.[58] In studies with myeloperoxidase-deficient polyphonuclear leukocytes, Lehrer and Cline demonstrated that myeloperoxidase is importantly involved in the killing of intracellular yeast, such as *Candida albicans,* and that bacterial killing was reduced in myeloperoxidase deficiency.[59,60] Klebanoff and his coworkers refined and extended these observations, and their findings underscore the wide range of microbicidal and cytocidal activity of the myeloperoxidase-H_2O_2-halide system.[4,49]

Despite these observations, the precise role of myeloperoxidase in distinction to that of other oxidative mechanisms in the normal antimicrobial activity of polymorphonuclear leukocytes needs clarification. This question is raised primarily because the antibacterial activity of myeloperoxidase-deficient polymorphonuclear leukocytes is only mildly impaired,[58,60,61] and that the incidence of genetic myeloperoxidase-deficiency is suprisingly common and may be as frequent as one in several thousand of the normal population.[61] Most affected individuals have little difficulty with infection unless they also have diabetes mellitus;[59,61] in these subjects the primary infectious complications are with *Candida* species.

It has been suggested that myeloperoxidase-deficient polymorphonuclear leukocytes may have highly effective compensatory mechanisms to kill bacteria which bypass the deletion in this enzyme.[4,58,62] Conversely, myeloperoxidase may only be required for the oxidative killing of specific

complex eukaryotic organisms, whereas the formation of O_2- and H_2O_2 are essential for prokaryotic cell killing. Both of these possibilities are the subjects of active investigation.

The Chediak-Higashi syndrome. This rare autosomal recessive disorder effects the formation of single-membrane bound organelles in many cells, including neutrophils, monocytes, and eosinophils. Patients have repeated infections with a variety of catalase-positive and negative bacteria.[63] Infectious complications appear to result from multiple abnormalities in neutrophils and monocytes, including neutropenia, impaired chemotactic responses, and delayed intracellular killing of ingested bacteria.[64,66] The giant lysosomes of Chediak-Higashi neutrophils contain enzymes and proteins of both azurophilic and specific granules.[67] Following phagocytosis, degranulation occurs in a selective and delayed fashion and killing of ingested bacteria is delayed to approximately the same extent as with myeloperoxidase-deficient cells. Oxidative responses are normal or exaggerated.[65] These observations suggest that the intraphagosomal formation of O_2- and H_2O_2 are essential for prompt intracellular killing of microorganisms, in particular, bacteria. In contrast, the delivery of lysosomal enzymes is perhaps most involved with the digestion or late killing of bacteria.[68]

Other Killing Mechanisms

Neutrophils possess powerful systems that kill bacteria by nonoxidative mechanisms. These include permeability-altering cationic proteins.[69,72] While these proteins are likely to play some role in intracellular destruction of ingested organisms,[73] at present it is difficult to assess their relative importance, as has been the case with the genetic disorders of oxidative-killing described above. The identification of inherited disorders of these proteins may help in this regard.

Therapy of Phagocytic Cell Disorders

Much has been learned about normal neutrophil amtimicrobial mechanisms from the study of patients with the genetic disorders described above. This information is being extended to eosinophils, monocytes and macrophages.[6,11] From the therapeutic standpoint, attempts at the correction or the improvement of normal neutrophil function during periods of sepsis are in their infancy. As mentioned, the administration of ascorbate[21] or levamisole[23] may benefit some patients with defects in neutrophil

chemotaxis. The provision of H_2O_2 *in vitro* has corrected the abnormal functions of chronic granulomatous disease leukocytes,[51,52] but the application of this strategy to patients awaits practical development.

Once patients with neutrophil disorders are identified and an increased prospensity to infection confirmed, primary efforts at treatment presently center on the use of specific antistaphylococcal antibiotics for some patients (hyper IgE syndrome, chronic granulomatous disease), trimethoprim-sulfanamides for others (chronic granulomatous disease),[5,74] and the prompt recognition and specific therapy of infection in all. Leukocyte transfusion therapy may help some patients during active infection who do poorly with antimicrobial treatment alone.[12] Bone marrow transplantation has been completed successfully in one patient with chronic granulomatous disease.[75] While specific treatment of most of these disorders is still a goal for the future, their continued identification and clarification of abnormal mechanisms are worthwhile endeavors, both to understand how normal cells work as well as to develop means to modify phagocyte function when desirable.

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