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CHAPTER 113

LEUKOCYTE ADHESION DEFICIENCY AND OTHER DISORDERS OF LEUKOCYTE MOTILITY

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1. *Recurrent bacterial or fungal infections of the skin or mucous membranes are prominent in patients with quantitative deficiencies of peripheral blood leukocytes. Such infections are also evident in patients with qualitative disorders resulting in insufficient accumulation of phagocytes at inflammatory sites. Among both patient groups, common pathogens such as* Staphylococcus aureus, Pseudomonas, *other gram-negative enteric species, or* Candida albicans *account for most infectious complications. Infected tissues in these patients are characteristically gangrenous or necrotic and devoid of pus and contain few granulocytes. Local inflammation may be minimal though the infection may lead to the destruction of cutaneous, subcutaneous, or submucosal tissues. A reliable interpretation of abnormal leukocyte functions assayed in vitro must take into consideration the clinical status of the individual patient. It is important to determine whether abnormal functions cause increased susceptibility to infection or simply reflect other factors surrounding the patient's condition such as pharmacologic agents, nutrition, infection, or underlying metabolic disease.*

2. *The molecular pathogenesis of a limited number of genetic or secondary disorders characterized by defective migration of leukocytes is known. Most notable is leukocyte adhesion deficiency, a heritable deficiency of the adherence proteins of the CD18 complex. This is a recently recognized autosomal recessive disorder characterized by recurrent bacterial infections, impaired pus formation and wound healing, and a wide spectrum of functional abnormalities in granulocytes, monocytes, and lymphoid cells. Superficial infections of body surfaces may invade locally or systemically. Typical small, erythematous, nonpustular skin lesions often progress to large, well-demarcated, ulcerative craters, or pyoderma gangrenosa, which heal slowly or with dysplastic eschars. Staphylococcal or gram-negative enteric bacterial organisms may be cultured from such lesions for up to several weeks despite antimicrobial therapy. Severe gingivitis and/or periodontitis is a major feature among all patients who survive infancy.*

3. *The recurrent infections observed in patients with leukocyte adhesion deficiency appear to reflect impairment of leukocyte mobilization into extravascular inflammatory sites. Infected tissues are devoid of neutrophils, even though marked peripheral blood leukocytosis (five- to twentyfold normal values) is a constant feature of this disorder. The severity of infectious complications appears to be directly related to the degree of glycoprotein deficiency. Two phenotypes, designated* severe *and* moderate, *have been identified. Severely deficient patients have essentially undetectable expression (<0.3 percent of normal amounts) of these glycoproteins on the surface of their leukocytes. Moderately deficient patients express 2.5 to 6 percent of normal levels. Patients with severe deficiency have either died in infancy or demonstrated a susceptibility to life-threatening systemic infections. In those with moderate deficiency (mean age 21 years, range 11 to 38 years) life-threatening infections have been infrequently observed.*

4. *Abnormalities of adhesion-dependent functions in leukocytes including chemotaxis and aggregation have been observed among all patients studied. Phagocytosis of iC3b-opsonized particles is deficient since one of the deficient glycoproteins is the receptor for complement component C3. Abnormalities of antibody-dependent cellular cytotoxicity have also been observed in several patients. In contrast, adherence-independent cellular functions including f-Met-Leu-Phe receptor-ligand binding, oxidative metabolism, degranulation mediated by soluble stimuli, and intracellular microbicidal activity are relatively normal in most patients. Overall, more profound functional abnormalities have been observed among severely deficient as compared with moderately deficient patients.*

5. *The molecular basis of leukocyte adhesion deficiency has been found to involve a recently characterized family of structurally and functionally related glycoproteins on the surface of myeloid cells. These glycoproteins are involved in a wide array of functions dependent on adhesion. Each consists of noncovalently associated α and β subunits with $\alpha_1\beta_1$ stoichiometry. They share an identical β subunit (M_r = 95,000) and are distinguished immunologically by distinct α subunits whose relative molecular weights are as follows: M_r = 165,000 for Mac-1α (αM), M_r = 177,000 for LFA-1α (αL), and M_r = 150,000 for p150,95α (αX). The World Health Organization designation for these glycoproteins is CD18 for β; CD11a for αL; CD11b for αM; CD11c for αX. In studies of six unrelated patients and four related patients and other members of their kindred the following five distinct variations in the β subunit were identified. (1) The subunit was undetectable. (2) The quantities of β-subunit mRNA and protein precursor were low. (3) An aberrantly large β-subunit precursor likely due to an extra glycosylation site was found. (4) An aberrantly small β-subunit precursor due to a polypeptide chain defect was found. (5) No gross abnormality in the β-subunit precursor was found. In studies of one kindred including four related patients of the moderate phenotype, a β precursor of identically abnormal small size was identified in each case. Heterozygotes in this family show both a normal and an abnormally small β precursor, and noncarriers show only the normal β precursor. Cell lines from patients synthesize normal α-subunit precursors, but in the absence of a normal β subunit, this precursor cannot associate in an $\alpha\beta$ complex, does not undergo carbohydrate processing, and is not expressed on the cell surface. The α subunit is apparently degraded in the absence of the β subunit.*

6. *Bone marrow transplantation with successful engraftment rendered unnecessary any further treatment in two patients. In two other patients, successful engraftment was achieved, but recov-*

ery did not occur either because of graft-versus-host disease or infectious complications. Transplantation is recommended for severely deficient patients because of the high incidence of death before the age of 2. Moderately deficient patients live longer but may also be susceptible to life-threatening infections. Therapeutic guidelines for management of moderately deficient patients are not well defined.

HISTORICAL ACCOUNTS

The important role of phagocytic cells in host defense was recognized a century ago by Elie Metchnikoff. Historic findings by this jobless Russian zoologist in 1882 were among the first scientific evidence of phagocyte-host defense interactions. Several years later Metchnikoff described this conceptual breakthrough as follows[1]:

> One day when the family had gone to a circus to see some extraordinary performing apes, I remained alone at my microscope, observing the life in the mobile cells of a transparent starfish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defense of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore in order to collect my thoughts.
>
> I said to myself that, if my supposition was true, a splinter introduced in to the body of a starfish larva, devoid of blood vessels or of a nervous system, should soon be surrounded by mobile cells as is to be observed in a man who runs a splinter into his finger. This was no sooner said than done.
>
> I was too excited to sleep that night in the expectation of the result of my experiment, and very early the next morning I ascertained that it had fully succeeded.
>
> That experiment formed the basis of the phagocyte theory, to the development of which I devoted the next twenty-five years of my life.

What Metchnikoff had described in the starfish experiment was not phagocytosis itself (i.e., the cellular ingestion of particles) but rather a more complex process involving the localized accumulation of cells at a point of injury. Further evidence that phagocytes undergo tropic migration was provided in 1888 by Theodore Leber, a German ophthalmologist.[2] He demonstrated for the first time migration of leukocytes toward chemical stimuli, a response later termed chemotaxis when observed in vitro. He wrote at that time[2]:

> The property of the leukocyte to tropic migration by substances foreign to the organism is of the greatest importance in making possible an extensive counteraction of the organism against external factors, since only in this way is the accumulation of a large number of leukocytes at the site of noxa assured.

Since the time of these early landmark observations, the critical and multifaceted functions of phagocytes in inflammation have been described in a voluminous scientific literature. However, an understanding of the clinical relevance of these many observations largely awaited the recognition of pathologic disorders in the functions of human phagocytes. In 1954, Janeway and associates[3] described a group of patients with severe recurrent soft tissue infections. Approximately a decade life, Quie and coworkers[4] demonstrated a profound defect of intracellular microbicidal activity of phagocytic cells from these patients, and Holmes and coworkers[5] subsequently demonstrated a defect of oxidative metabolism in this disorder, now termed *chronic granulomatous disease of childhood*. Subsequent to the recognition of this syndrome, a rapid succession of reports[6–11] described a heterogeneous group of patients with severe, recurrent sinopulmonary infections and/or staphylococcal soft tissue abscesses. Abnormal or diminished motility of neutrophils and/or monocytes was suggested as a major pathogenic mechanism accounting for these infectious complications. More recent investigations have successfully determined a molecular lesion accounting for or contributing to disturbed cellular adherence or motility in a limited number of clinical disorders. The scope of this chapter includes only those disorders for which molecular pathogenic mechanisms have been described or proposed. As background for these descriptions, selected aspects of the physiology of adherence and motility are discussed. Disorders of leukocyte killing are described in Chap. 114.

PHYSIOLOGY OF LEUKOCYTE LOCOMOTION

Definitions

The locomotion of leukocytes is regulated by a complex cellular apparatus responsive to many environmental stimuli. Among these are physical properties of substrates to which leukocytes adhere as well as chemical substances of host or microbial origin which influence the nature of migration.[12–15] Leukocytes demonstrate little movement in vitro in the absence of specific stimuli. Chemical substances, including chemotactic factors, may modify substantially the speed and direction of locomotion. Stimulated migration with no directional component is termed *chemokinesis* or *random locomotion*.[13] When under the influence of concentration gradients of chemotactic factors, the net direction as well as speed of a migrating cell population will be altered. The result is *chemotaxis*.[14–16] A positive chemotactic response results in the attraction of cells toward the stimulating agent with individual leukocytes, demonstrating a rather uniform orientation toward the origin of the chemotactic gradient.[17,18] It should be emphasized that the terms *random locomotion, chemokinesis,* or *chemotaxis* should be used only in cases where these forms of behavior have been demonstrated experimentally.

Methods to Evaluate the Motility of the Leukocytes In Vitro

A variety of techniques have been utilized to evaluate the migratory properties of myeloid or lymphoid cells. Evaluations of the kinetics and extent of the mobilization of leukocytes in various tissues of human subjects are limited to histopathologic assessments and applications of "skin window" techniques initially described by Rebuck and Crowley.[4,4a] Findings of large numbers of one or more types of leukocytes in tissue exudates does not exclude the possibility that these cells infiltrated too late to prevent the establishment of infections.[19] The skin window technique and several more quantitative modifications[20] have proved of limited value for several reasons. Difficulties encountered in creating uniform skin lesions in addition to the pain or possible infectious complications as-

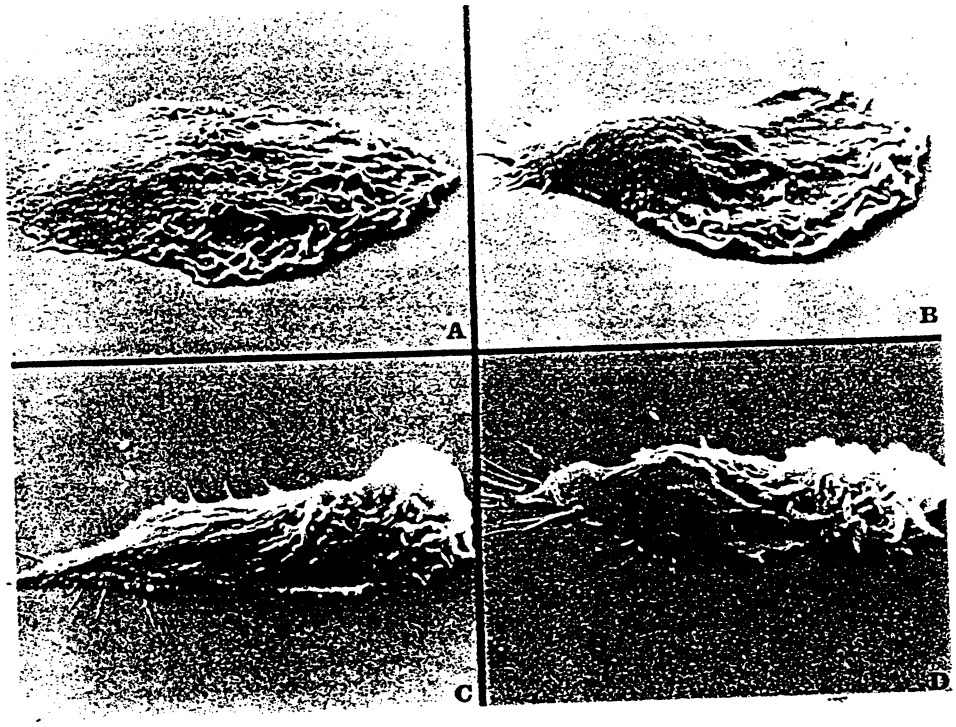

Fig. 113-1 Scanning electron micrographs of neutrophils responding to chemotactic factor. Human neutrophils were exposed to a gradient of the chemotactic factor, f-Met-Leu-Phe. Within 60 s of sensing the chemotactic factor, the cell spreads on the albumin-coated glass surface and ruffles on the side of the cell adjacent the higher concentration of the chemotactic factor (*A*), extends the lamellepodium forward and begins to develop a uropod with retraction fibers (*B*), elongates forming more pronounced ruffles within 2 to 3 min (*C*), and continues to migrate toward increasing concentrations of the chemotactic factor at a rate of between 5 and 15 μm/min (*D*). During migration the front of the cell is constantly ruffled and the tail of the cell (uropod) has a much smoother surface than the attached retraction fibers. (*By permission of D.C. Anderson et al., Cell 31:719, 1982.*)

sociated with the use of abrasive techniques limit their overall suitability for clinical application. Evaluations of the migratory functions of leukocytes in clinical samples are most commonly performed in vitro.

Visual Assays. Direct observations of individual cells undergoing locomotion in vitro can provide quantitative as well as descriptive information.[16,17,21] These assessments may be embellished by time-lapse photomicrographic recordings. Leukocytes, particularly neutrophils, are relatively fast moving cells compared, for instance, with fibroblasts. Most descriptions of the morphology of leukocytes during locomotion in vitro indicate that they assume a characteristic bipolar shape[17,17a] (Fig. 113-1). This involves the appearance of a veil-like, flattened membrane, or lamellipodium, at the anterior end of the cell. This pseudopod does not contain cytoplasmic organelles which remain in a more sharply delineated posterior portion of the cell. Migrating cells also generally demonstrate a uropod or taillike structure with retraction fibers developing as the cell migrates. During locomotion this oriented morphology is retained. Cells elongate as lamellipodia spread forward and cytoplasmic contents stream into the anterior portion of the cell. As they translocate, neutrophils continuously develop new lamellipodia which adhere to the surface upon which the cells crawl.

To study the effects of chemotactic factors on these events, it is necessary to establish experimental conditions which allow exposure of cells to stable and continuous chemical gradients. Under such conditions, effects on both the rate and direction of cell orientation or movement can be observed. Most investigators have used microbial point sources such as bacterial clumps or spores of yeast, or specially designed orientation chambers for applications in visual assays.[15–17,21]

The bipolar shape seen in migrating cells also occurs if neutrophils[22] and monocytes[23] are exposed to chemotactic stimuli in suspension. Thus, the cells need not be attached to a surface in order to assume a bipolar shape. Using this morphologic change as a sensitive indicator of chemotactic stimulation, abnormal cellular responses have been identified in clinical conditions characterized by increased infections, and the inhibitory action of selected pharmacologic agents has been assessed.[24–26]

Micropore Filter and Agarose Assays. A second group of assays of chemotaxis include those in which the effect of chemoattractants on locomotion of a population of cells, or a sample of a population, is measured. In these assays, no attempt is made to follow the paths traveled by individual cells, and measurements are made generally after experimentally stopping cell migration. Each of these assays, in principle, represents modifications of the micropore filter method[22] originally described by Boyden[27] in which a test cell population migrates into or through a micropore filter toward a test reagent placed in an adjacent stimulant compartment. Since this assay was easy to use and gave reasonably quantitative measurements of the movement of cell populations, it rapidly gained widespread clinical applicability. It became possible to make comparisons between the chemotactic responses of cells from patients and those of control subjects. An important modification of the micropore filter method[17] distinguishes chemotaxis from chemokinesis. This is accomplished by the use of protocols in which a range of concentrations of a stimulant reagent is incorporated independently in the cell and stimulant compartments of a culture chamber.[28] A conceptually similar technique in which leukocytes migrate toward a chemoattractant source on plastic or glass under agarose[29] has also proved useful.

These various assays have advantages as well as disadvantages. Visual assays allow detailed examinations of the morphologic events of moving cells and the influences of attractants, inhibitors, or drugs. By analysis of the paths taken by individual cells, a detailed description of the influence of these agents on cellular velocity, direction of movement, turning behavior, adhesiveness, and other cellular properties can be obtained. A disadvantage of visual assays is the difficulty quantifying cell responses to a given agent, i.e., to obtain

ose-response curves. In contrast, dose-response curves are asily obtained with filter assays which have proven useful for ıe study of the attractant or inhibitory activity of unknown ıctors. Since these assays give different types of information nd neither gives a complete picture of the locomotion of leucocytes, it is reasonable to suggest the use of both techniques ı parallel in studies of patients with suspected defects of lomotion.

ensory Mechanisms of Cellular Locomotion

arly experimental observations of leukocytes undergoing hemotaxis suggested the existence of sensory mechanisms by hich cells are able to detect differences in concentrations of hemotactic molecules in their environment. Identification of ighly adaptive sensory mechanisms for neutrophils as well as ther types of leukocytes resulted largely from investigations f the nature of chemotactic factors and the specific cellular eceptors for these chemotactic moieties.[30]

Early studies documented that the activation of serum comlement liberates highly active endogenous chemotactic factors or neutrophils and other leukocyte cell types and that this ctivation provides an important mechanism by which neutrohils and monocytes are mobilized into inflammatory lesions ı vivo.[31-34] Other well defined chemoattractants more reently described include products of bacterial protein synthesis (e.g., *N*-formylated-methionyl peptides),[35,36] the arachilonate metabolite, leukotriene B_4 (5,12-dihydroxyeicosatetraıoic acid),[37] platelet-activating factor,[38] and a lymphokine, ermed *lymphocyte-derived chemotactic factor*, produced by anigen or mitogen stimulated lymphocytes.[39] Mast cells contain etrapeptides which are chemotactic for eosinophils and are eleased upon exposure of mast cells to specific antigen.[40] A ell-derived chemotactic factor produced by neutrophils after ngestion of crystalline materials such as monosodium urate or alcium pyrophosphate has been also described.[41]

The cellular nature of an inflammatory tissue exudate is hought to reflect the net influences of one or more chemotacic moieties (and/or inhibitors) of microbial and host origin enerated locally at inflammatory sites. While mobilization of ıeutrophils is more frequently associated with non-IgE imıune complexing or microbe-complement interactions, the osinophil predominates in both the early and late cellular inıltrates of immediate-hypersensitivity reactions. While factors hemotactic for neutrophils are released by IgE-dependent ıctivation of mast cells and mast cell-rich tissues, the predominant chemotactic activity of anaphylaxis favors eosinohils and is attributable to low molecular weight chemotactic factor.[40] Although delayed hypersensitivity reactions may manifest infiltrates containing multiple leukocyte popuıations, they are characteristically devoid of neutrophils. This may be related to potent inhibitors that are produced by immunologically stimulated lymphocytes and block accumulation of neutrophils.[42]

The binding of chemotactic factor to an appropriate receptor initiates a series of coordinated biochemical and cellular events, some of which contribute to cellular locomotion. These include alterations in ion fluxes[43,44] and transmembrane potential,[45] alterations of cell shape and adhesiveness,[22] secretion of enzymes from intracellular granules,[30] production of superoxide and other highly energized oxygen radicals,[30-46] and a stimulation of energy metabolism via enhancement of transmembrane glucose transport and anaerobic glycolysis.[47] These various functions are probably sequentially activated and highly integrated in the inflammatory response. Secretory and oxidative functions in vitro are generally elicited by much higher concentrations of chemotactic factors than those optimal for chemotaxis. Thus, in a graded fashion, locomotion into a chemotactic gradient in vivo may precede secretory and oxidative events which ultimately contribute to microbicidal or cytotoxic functions required in localized inflammatory sites. Recent evidence suggests that the diverse functional responses of neutrophils to chemotactic stimuli reflect complex regulatory influences of the chemotactic factor receptors and the associated cellular transduction pathways.[30,48-53]

The peptides derived from the C5 component of complement, C5a and $C5a_{desarg}$, represent the most important of the complement-derived chemotactic factors.[31-34] C3a cleaved from C3 by the action of trypsin or C3 convertase was reported to be chemotactic for neutrophils in early studies, but later investigations failed to confirm these findings and indicated that the biologic activity of C3a was primarily anaphylatoxin. Human C5a is a 74-residue glycosylated peptide (M_r = 9000) produced by proteolysis of C5 by C5 convertase generated by activation of either the classic or alternate complement pathways or by intracellular granule associated proteases of neutrophils which act directly on C5.[54] The carboxyl terminal arginine residue of C5a formed in serum is rapidly hydrolyzed by carboxypeptidase N, to produce a less potent but more stable moiety, $C5a_{desarg}$.[34] $C5a_{desarg}$ lacks anaphylatoxin activity and is ten- to twentyfold less active than C5a as a chemoattractant for neutrophils. There is evidence that $C5a_{desarg}$ requires the presence of a naturally occurring anionic polypeptide (termed *$C5a_{desarg}$ cochemotaxin*) is serum or plasma, for full chemotactic activity.[55,56] Much of the chemotactic activity of zymosan activated serum is due to $C5a_{desarg}$ acting together with this cochemotaxin.

All C5a-mediated functions (including chemotaxis, degranulation, or superoxide generation) depend on binding of C5a peptides to a specific cell surface receptor in a rapid, specific, and saturable fashion.[57] Early studies employing [^{125}I]C5a showed that rapid and saturable binding of this moiety to neutrophils was essentially complete within 3 to 5 min. Approximately one-half saturation occurred at a C5a concentration of 3×10^{-9} *M*, and the number of binding sites per cell was estimated to be 1 to 3×10^5. Studies of the binding of radio-labeled $C5a_{desarg}$ to neutrophils have not been reported. However, $C5a_{desarg}$ has been shown to be approximately 400-fold less potent than C5a with respect to its ability to compete with radiolabeled C5a for the same binding site on neutrophils.[58] The critical importance of C5a and $C5a_{desarg}$ as chemotactic signals in vivo is related to the fact that theoretically these moieties may be generated continuously at the gradient source in inflammatory lesions. In fact, it has been demonstrated experimentally that renewable chemotactic gradients are established by suspending test microorganisms in serum.[21]

A group of *N*-formyl methionyl dipeptides was found to possess chemotactic activity for neutrophils.[35] Later studies[59] demonstrated that formyl tripeptides such as f-Met-Leu-Phe are extremely active, effecting maximum chemotactic responses at concentrations of 10^{-9} to 10^{-10} *M*, and several investigators demonstrated specific saturable binding of these peptides to the surface of neutrophils or neutrophil membranes. The binding of formyl norleucyl-Leu-[^{3}H]Phe to rabbit peritoneal neutrophil membranes was found to have a K_d of 1.5×10^{-9} *M*, and there were approximately 10^5 binding

sites per cell.[60] Later studies of human neutrophils indicated that *N*-formyl peptides bind with high affinity to a definable number (approximately 40,000 to 60,000) of receptors on the neutrophil surface.[61,62] Specific saturable receptors for *N*-formylated peptides have also been detected on the surface of human monocytes[63] and guinea pig peritoneal macrophages.[64] The ability of a series of synthetic *N*-formylated peptides to compete for f-Met-Leu-[^{3}H]Phe neutrophil binding paralleled the potency of these peptides in eliciting biologic responses indicating that the *N*-formyl peptides share a common receptor.[59] Although the formyl peptides are synthetic molecules, they are thought to represent analogues of products of bacteria or other microorganisms. Bacteria such as *Escherichia coli* begin protein synthesis with formyl methionine which is later cleaved from the protein chain. Formyl-methionine is not used in eukaryotic protein synthesis except in mitochondria. Thus, the formyl-methionyl peptides likely represent a characteristic prokaryotic product which neutrophils can distinguish from products of host origin.[65] In a manner similar to other true chemotactic factors, formyl methionyl peptides elicit a wide spectrum of responses. Thus, they have proven to be ideal for studies of cellular physiology.[30,66]

Biochemical characterizations of formyl peptide receptors on leukocytes have utilized a variety of covalent cross-linking techniques and have demonstrated that the formyl peptide receptor is a glycosylated protein (M_r = 60,000 to 70,000).[67–69] The receptor has been purified from an immortal myeloid cell line (HL60) and reconstituted into phospholipid vesicles.[70] Removal of carbohydrate from the receptor yields a protein (M_r = 32,000) which maintains ligand binding capacity.[71] Following ligand binding to neutrophils, f-Met-Leu-Phe-receptor complexes are rapidly internalized and translocated to a Golgi-rich fraction.[30]

Many lipids and fatty acids have weak stimulatory effects on locomotion of neutrophils and mononuclear phagocytes, although in many cases these have not been shown convincingly to be chemotactic effects. Early studies indicated that oxidized derivatives of arachidonic acid, and later, 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid attracted neutrophils into filters in chemotaxis chambers.[72] More recent studies demonstrated that other hydroxy products of lipoxygenase-modified arachidonic acid are chemoattractants. The most potent and well characterized of these is 5(*S*),12(*R*)-dihydroxyeicosa-6-14-*cis*-8,10-*trans*-tetraenoic acid (leukotriene B_4). Leukotriene B_4 is generated by the 5-lipoxygenase and related enzymes of neutrophils, macrophages, and mast cells and represents the major endogenous lipid chemoattractant secondarily released as a result of cellular activation by exogenous chemotactic factors.[52] At nanomolar concentrations, leukotriene B_4 elicits chemotactic and chemokinetic migration, and at much higher concentrations it stimulates release of lysosomal enzymes, superoxide production, enhancement of adherence, expression of C3b receptors, and complement-dependent cytotoxicity by neutrophils.[52,53,73,74] Stereospecific binding sites representing functional receptors for leukotriene B_4 have been identified on human neutrophils localized primarily in the plasma membrane.[53,74,75] Two classes of receptors for leukotriene B_4 are expressed. The high affinity (K_d = 0.4 n*M*) receptors appear to be coupled to chemotactic migration, and low affinity (K_d = 61 n*M*) receptors appear to be coupled to secretory functions such as enzyme release.[52]

Several lines of investigation indicate that receptors for chemotactic factors are qualitatively and quantitatively influenced by complex cellular regulatory processes which are operative following receptor-ligand binding. Both high and low affinity receptors for f-Met-Leu-Phe and leukotriene B_4 on human neutrophils and macrophages have been characterized.[50,52,76,77] Increasing evidence suggests that at least a portion of high and low affinity receptors are interconvertible through processes functionally linked to a pertussis toxin-sensitive regulatory *N* protein and guanosine nucleotides.[50] Rapid internalization of f-Met-Leu-Phe-receptor complexes appears to represent one mechanism by which activated cells are functionally down-regulated upon exposure to high concentrations of chemotactic factors.[78,79] Conversely, treatment of neutrophils with f-Met-Leu-Phe or agents which induce exocytosis of specific granules may increase the number of f-Met-Leu-Phe receptors on the cell surface.[30,50,80–82] These additional receptors are derived from incompletely defined intracellular pools which may be associated with specific granules and may serve to replace internalized receptors during cell locomotion.[83] The physiological significance of modulations in chemotactic receptors in vivo is uncertain, but these events may allow for selective adaptive amplifications or attenuations of one or more cellular responses during inflammation.

Adhesive Determinants of Cellular Motility—Functional Studies

Adherence is of central importance in a wide spectrum of functions in granulocytes, monocytes, and lymphocytes.[84] For example, the precise nature of cellular interactions with various surfaces has been shown to influence the mobility of neutrophils and monocytes in vitro.[22,85] On a two-dimensional surface such as glass or plastic, the physical interactions with the substratum upon which leukocytes or other cell types crawl influence the extent and direction of cell migration.[22,85–87] The following evidence supports this concept: (1) A Mg^{2+}-deficient medium diminishes both cell migration and adherence, and neither effect is corrected by addition of exogenous Ca^{2+}.[43,90,91] (2) Substrates to which cells irreversibly adhere (e.g., neutrophils adhering to uncoated glass) or very minimally adhere (e.g., neutrophils on polypropylene plastic) effectively impair cell translocation even in the presence of chemotactic gradients. (3) Some cell types can crawl up a gradient of adhesiveness but cease to migrate when adherence becomes high.[89] (4) The chemokinetic influence of albumin, fibrinogen, or other serum proteins employed in visual chemotactic assays is linked to their reduction of cell-substrate adhesion.[92–94] Thus, cell migration on two-dimensional surfaces (as in the visual chemotaxis assays) requires adhesion that is sufficiently strong to allow attachment to a substratum but weak enough to permit continued locomotion. Enhancement of adherence to artificial substrates,[22,24,85] endothelial cells,[85–97] or other cell types[98–100] occurs when neutrophils are exposed to a chemotactic stimulus. With optimal levels of the stimulus, motility is augmented, but high concentrations may result in reduced motility, especially on artificial surfaces in vitro.[85,98,101]

Some investigators have presented experimental evidence that neutrophils can move relatively independently of adherence in certain instances.[102–105] Their results indicate that movement through three-dimensional matrices (e.g., cellulose filters used in the micropore filter assay for chemotaxis, or collagen gels) is largely adherence-independent. Though adherence-independent locomotion has received little

study, it may well be important in migration of leukocytes through connective tissue after they leave the vascular system.[102,104]

Molecular Mechanisms in the Adhesiveness of Leukocytes

The molecular events contributing to cellular adherence are incompletely defined. Observations in vivo have revealed that neutrophils and monocytes adhere avidly and preferentially to vascular endothelium adjacent to a site of inflammation before their migration into tissues.[4,106] Since this adherence appears to be necessary for the localized recruitment of leukocytes into extravascular inflammatory sites, many investigators have examined the mechanisms by which the adhesiveness of neutrophils and monocytes are modulated by inflammatory stimuli.

A secretory mechanism by which adherence-promoting constituents are rapidly mobilized to cellular surfaces in response to chemotactic receptor occupancy represents one attractive explanation for rapid modulation of adherence. The process of extracellular release or exocytosis of specific granules or other secretory vesicles appears to be a highly integrated mechanism by which cell surface adherence properties are altered in response to inflammatory stimuli.[107] The perigranular membrane of specific granules seems to provide a source of new membrane required for morphologic alterations of motile cells.[108] Some experimental evidence suggests that specific granules also represent an intracellular pool from which the f-Met-Leu-Phe receptor[80,82] and glycoproteins with adhesive properties[109–114] are brought to cellular surfaces by chemotactic or secretory stimuli. The physiological and potential pathologic contributions of secretory-dependent functions of neutrophils have been emphasized by studies of patients with genetic deficiency of neutrophil specific granules.[83,115–117] Chemotactic factors also seem to stimulate a reduction in the negative surface charge of human neutrophils which has been at least temporally related to increased adhesiveness.[118–121]

Many different proteins on the leukocyte's surface participate in cellular functions dependent on adherence. Specific recognition of opsonized microorganisms is facilitated by membrane receptors for immunoglobulin G (IgG) and C3-derived ligands, which mediate adhesion of opsonized microorganisms before endocytosis is triggered.[122] Adhesion can be mediated by antibodies (IgG) bound to tissue cells through receptors on the surface of leukocytes for the Fc portion of the antibody molecule. This reaction activates killing of tissue cells to which the antibody is bound.[4,123] In addition, a recently characterized family of structurally and functionally related glycoproteins found on the surface of myeloid cells[124] is involved in a wide array of functions dependent on adhesion. The nomenclature, structure, cellular distribution and function of these glycoproteins are summarized in Table 113-1.[84,124,125] Each of these glycoproteins consists of noncovalently associated α and β subunits with $\alpha_1\beta_1$ stoichiometry. They share an identical β subunit (M_r = 95,000) and are distinguished immunologically by distinct α subunits whose relative molecular weights are as follows: M_r = 165,000 for Mac-1α(αM); M_r = 177,000 for LFA-1α(αL); and M_r = 150,000 for p150,95α(αX).[125] The World Health Organization designations for these glycoproteins are CD18 for β; CD11a for αL; CD11b for αM; and CD11c for αX.[126] That these glycoproteins function in cellular adhesion was initially shown by the ability of specific monoclonal antibodies to inhibit a wide variety of granulocyte, monocyte, and lymphocyte functions dependent on cell-substrate or cell-cell interactions.[84] Soon after this family of glycoproteins had been defined with monoclonal antibodies, an inherited deficiency was recognized in humans.

Mac-1, LFA-1, and p150,95 have different roles in adhesion. Mac-1 is a complement receptor (CR-3) which binds the ligand iC3b.[127,128] Monoclonal antibodies to Mac-1 inhibit binding and phagocytosis in iC3b opsonized particles by neutrophils and macrophages.[129,130] Purified Mac-1 binds iC3b,[127] and binding is dependent on Mg^{2+}. Mac-1 mediates adherence of neutrophils to endothelial cells and a variety of other substrates, and thus participates in numerous cellular functions including migration on surfaces to which leukocytes must attach in order to crawl, accumulation of neutrophils at sites of inflammation, phagocytosis of foreign particles such as latex beads, secretion of granule associate enzymes that results from

Table 113-1 The Mac-1, LFA-1, p150,95 Glycoprotein Family

	Mac-1		*LFA-1*		*p150,95*	
Subunits	*αM*	*β*	*αL*	*β*	*αX*	*β*
Molecular weight	170,000	95,000	180,000	95,000	150,000	95,000
WHO designation	CD11b	CD18	CD11a	CD18	CD11c	CD18
Cell distribution	Monocytes Macrophages Granulocytes Large granular lymphocytes		Lymphocytes Monocytes Granulocytes Large granular lymphocytes		Monocytes Macrophages Granulocytes	
Chemotactic or secretory stimulation increases surface expression	Yes		No		Yes	
Functions inhibited by monoclonal antibodies	Complement receptor type three function (iC3b binding, phagocytosis and intracellular killing of C3 opsonized microorganisms); granulocyte adherence, apreading, aggregation, ahemotaxis, and antibody-dependent cellular cytotoxicity		Cytolytic T lymphocyte-mediated killing and T-helper cell responses Natural killing Antibody-dependent cellular cytotoxicity Phorbol ester-stimulated lymphocyte aggregation		Granulocyte adherence and aggregation	
Common features	The β subunits appear identical. The α subunits αM and αL are 35% homologous in sequence. The α and β subunits are noncovalently associated in $\alpha_1\beta_1$ complexes. Both α and β subunits are glycosylated and expressed on cell surface. All functions shown require divalent cations.					

attachment of neutrophils to a surface, and cytotoxicity associated with attachment of neutrophils to other tissue cells.[86,123,129,131–133] LFA-1 participates primarily in lymphocyte and monocyte adhesion.[134,216,217] It can mediate antigen-independent adhesion and also appears to be required for T-lymphocyte antigen-dependent adhesion to and killing of some target cells. Similarly, LFA-1 has been demonstrated to be important in natural killing and antibody-dependent killing by lymphocytes and neutrophils, and in T-lymphocyte helper cell interactions.[123,133,135,136] As is true for Mac-1-dependent functions, all LFA-1-dependent cellular functions require Mg^{2+}.

The functional role of p150,95 has been demonstrated only recently. It serves as a receptor for the complement component iC3b on macrophages and neutrophils.[127,137,137a] On monocytes, p150,95 mediates adherence to endothelial cells, tumor cells, and other substrates.[138] Monocyte extravasation and differentiation into tissue macrophages is accompanied by increased p150,95 expression,[139] as is differentiation of myelomonocytic precursor cells in vitro.[140] On lymphoid cells, p150,95 is a marker of cell activation. Although most cytolytic T-lymphocyte clones express higher amounts of LFA-1 than p150,95, a subset expresses equal quantities, and functional studies show that both p150,95 and LFA-1 mediate attachment of lymphocytes to target cells.[141]

Biosynthesis of these glycoproteins has been studied both in the mouse and human,[124,142] and translation and glycosylation have been studied in the mouse in vitro.[143] Using cloned probes, the murine Mac-1 α-subunit mRNA of 6 kb[144] and the human β-subunit mRNA of 3.2 kb[145] have been defined. Thus, it now appears that each of the three α subunits and the common β subunit are encoded by a separate mRNA. These subunits are synthesized as precursors which are cotranslationally glycosylated with *N*-linked high mannose carbohydrate groups. After α- and β-subunit association, which occurs 1 to 2 h after synthesis, most of the high mannose groups are converted to complex-type carbohydrates in the Golgi apparatus, and the subunits increase slightly in molecular weight. The mature glycoproteins are then transported to the cell surface or to storage sites in intracellular secretory vesicles.

In unstimulated neutrophils and monocytes, Mac-1 and p150,95 are present in one or more intracellular compartments as well as on the cell surface.[110,146] Inflammatory mediators including C5a and f-Met-Leu-Phe stimulate a four- to tenfold increase in Mac-1 and p150,95 (but not LFA-1) on neutrophil or monocyte surfaces.[131,132,147] Increased surface expression (as shown by monoclonal antibody binding in flow cytofluorography) is near maximal within 10 minutes at 37°C and is not impeded by protein synthesis inhibitors.[146] Intracellular pools of Mac-1 and p150,95 are associated with one or more types of cytoplasmic granules.[109,117,148] It is likely that the biosyntheses of Mac-1 and p150,95 destined for the cell surface and for intracellular storage sites are similar.

In recent studies, the individual contributions of Mac-1 subunits to adherence-dependent functions of neutrophils have been delineated utilizing normal neutrophils treated with monoclonal antibodies.[129] Phagocytosis of particles selectively opsonized with C3-derived ligands, and binding of iC3b opsonized sheep red blood cells are generally inhibitable by anti-αM but not by anti-β, -αL, or -αX. Monoclonal antibodies to αM, αX, and β inhibit adherence of neutrophils, and relatively more inhibition is observed when increased surface expression of αMβ and αXβ complexes is stimulated by inflammatory mediators. Both stimulated and baseline adherence is almost completely inhibited by monoclonal antibodies to the β subunit and the Mac-1α subunit. The general order of potency of inhibition is anti-β > anti-Mac-1α > anti-p150,95α > anti-LFA-α which reflects the relative amounts of each molecule expressed on the surface of neutrophils. Inhibition of chemotactic migration by anti-β, or anti-αM antibodies is observed in the subagarose assay (two dimensional) but not micropore filter assay (three dimensional) where adherence plays a relatively minor role. Inhibition is dependent on a continuous cell exposure to anti-Mac-1α or anti-β during the assay suggesting that recycled or new Mac-1 is required continuously for chemotaxis. These findings suggest that the Mac-1 molecule has adhesive properties in addition to a specific capacity to recognize and bind iC3b. Notably, none of the monoclonal antibodies to Mac-1 demonstrates inhibitory effects in assays of functions which do not depend on adherence such as enzyme release in response to chemotactic stimulation. Such functions are also normal in cells genetically deficient in these glycoproteins.

The Role of Chemotaxis in Inflammatory Reactions

The possibility that chemotaxis represents the principle mechanism for mobilizing leukocytes into inflamed tissues remains hypothetical. Most studies in vivo have failed to demonstrate directional migration of cells in extravascular loci.[149–151] Possibly this is true because most leukocytes exit vessels in or near the center of an inflammatory lesion and, therefore, in or near the center of any chemotactic gradient which may be operative.[152] Further, stable chemotactic gradients necessary for demonstration of directional migration in vitro may not occur in vivo or may exist over extremely small distances given the dynamic nature of the acute inflammatory response.

Though directional migration in vivo has not been clearly shown, considerable evidence indicates an important role for chemotactic factors in the recruitment of neutrophils, monocytes, or other leukocyte types into extravascular inflammatory sites. Studies of the physiological turnover of neutrophils indicate that very small numbers of cells are detectable in uninflamed tissues.[153–156] This is in striking contrast to the rapid and intense infiltration of neutrophils into tissues during acute inflammation. In animal models, $>10^7$ neutrophils per hour can accumulate in skin sites receiving an intradermal injection of a chemotactic factor; peak accumulation occurs within 1 to 4 h with an abrupt decline thereafter.[156] The entry of monocytes into inflamed rabbit skin parallels the kinetics of neutrophils for the first 4 to 6 h but is maintained thereafter for up to 20 h.[156–157] Injections of chemotactic factors including leukotriene B_4, platelet activating factor, C5a, or f-Met-Leu-Phe into rabbit skin elicit a significant neutrophil infiltration of the injection site.[157,158] Several other studies have documented a temporal association of chemotaxigenesis and the influx of neutrophils, monocytes, or macrophages into experimental inflammatory lesions mediated by immune complexes, glycogen, or chemotactic factors.[32,106,159,160]

Interactions of Leukocytes with Endothelium

Among the morphologic events that must be considered in order to understand the process of leukocyte migration in vivo are: (1) the margination of leukocytes occurring especially in the postcapillary venules, (2) the migration of leukocytes be-

tween endothelial cells, (3) the penetration of the vascular basement membrane by leukocytes, and (4) the migration into extravascular tissues. Although these events are incompletely understood, recent studies have provided considerable new insight into the molecular basis of at least the initial interactions of leukocytes with vascular endothelium.

A consistent observation in microscopic studies in vivo has been that marginated neutrophils roll along the endothelium of postcapillary venules for considerable distances before detaching, stopping, or emigrating through the blood vessel wall.[106,161–163] It appears that the endothelium becomes sticky[164,165] adjacent to a site of inflammation.[84,106,153,166] Localized adhesiveness of leukocytes to endothelium could occur as a result of: (1) local changes in the endothelium, (2) changes in the leukocyte, and/or (3) local changes in blood flow and, coincidentally, shear forces, shifting the balance in favor of intercellular adhesion. Since the endothelium is part of the permanent architecture of an inflamed tissue and the leukocyte only a passerby, many investigators have assumed that alterations of endothelial adherence play a primary role in the localized or directed attachment of leukocytes at these sites. Several lines of investigation, however, have shown that alterations in adhesiveness of circulating leukocytes resulting from systemic or localized exposure to chemotactic factors play an important active role. It seems likely that synergistic changes in both leukocytes and endothelial cells result from exposure to inflammatory stimuli.

Under steady-state conditions in the normal human adult, approximately 2.5×10^{10} circulating neutrophils and another 2.5×10^{10} marginated neutrophils are contained within the bloodstream. Although minor shifts between marginated and circulating pools represent physiological events, it has been recognized that rapid marked changes in the white blood cell count can be induced by major shifts between the circulating pool and the marginated pool in a number of pathologic states. Epinephrine induces neutrophilia by increasing the circulating pool at the expense of the marginal pool. This shift is associated with a transient decrease in the adhesiveness of neutrophils. A transient neutropenia can be induced experimentally by endotoxin, gelatin, dextran, cobra venom factor, or contact of blood with a foreign surface as during renal dialysis or continuous flow filtration by leukapheresis.[167–169] In each case there is some intravascular complement activation with generation of chemotactic factors (e.g., C5a). In animal models, infusion of complement or other chemotactic moieties induces a rapid transient neutropenia.[170] Neutropenia observed in clinical conditions characterized by intravascular complement activation (and/or the presence of other chemotactic factors) is thought to result from increased adherence of circulating neutrophils coincident with an increase in marginated pools and/or sequestration of leukocytes in target organs. Chemotactic factors elicit rapid alterations in adhesiveness of neutrophils and monocytes for endothelial cells in vitro, while no direct effects of chemotactic agents on endothelial adhesiveness have been observed in most studies.[22,95,96,171–177]

Blood flow may have several influences on leukocyte accumulation in inflammatory sites. Many vessels that are normally closed become open to flow in inflammation, and some evidence suggests that an initial slowing of flow in small vessels in inflamed tissues may reduce shear forces which normally diminish leukocyte-endothelial interactions. However, enhanced adherence of leukocytes to endothelium in inflamed sites has been observed without slowing of flow.[106] The well documented predilection of neutrophils to attach to high venular endothelium in postcapillary venules may, in fact, be related to reduced shear forces, since the postcapillary venule is the site of the first major decrease in vessel wall shear stress.[178,179] There is no direct evidence that neutrophils show a predilection for adherence to any particular endothelium. Preferential sequestration of neutrophils in the lung in complement activation states may reflect the fact that the lung is the initial capillary bed encountered by these activated cells within the venous circulation rather than a specific homing to pulmonary microvasculature. In contrast, circulating lymphocytes demonstrate considerable specificity with respect to high endothelial veins of peripheral or gut associated lymphoid tissues or other target organs. Recent observations indicate that specialized surface molecules of recirculating B or T lymphocytes are involved in this specificity.[180–185]

The important role of the CD18 complex of glycoproteins in the mediation of leukocyte-endothelial interactions in vivo has been emphasized by observations that neutrophils and monocytes which are genetically deficient in this complex fail to infiltrate into extravascular inflammatory sites despite the occurrence of profound granulocytosis in these patients.[131,132] The infusion of monoclonal antibodies to the β subunit into rabbits promotes a striking neutrophilia and severely inhibits exudation into experimental lesions (endotoxin coated sponges) in these animals.[186] These findings presumably reflect the same inhibitory effects of monoclonal antibodies to αM or β on stimulated adhesion of neutrophils or monocytes to endothelial monolayers in vitro.[174,187] The role of these glycoproteins in margination is uncertain since patients deficient in the CD18 complex demonstrate demargination responses to intravenous epinephrine, and experimental animals given intravenous infusions of monoclonal antibodies to the β subunit exhibit the same degree of marginating neutrophils rolling along venular endothelium as untreated animals.[188,189]

The role of one specific granule constituent in mediating adherence of neutrophils to endothelium has also been proposed. Purified lactoferrin, a prominent constituent of specific granules in neutrophils, promotes adherence of neutrophils to endothelial monolayers in vitro and, after intravenous infusion, produces neutrophil margination in rabbits.[111,112] Studies in some but not all patients with deficiency of specific granules and consequently lactoferrin have demonstrated abnormal leukocyte-endothelial interactions.[83,115,116]

The influences of a variety of inflammatory mediators on the adhesive and other properties of endothelium in vitro have been well documented in a number of laboratories.[190–193] Incubation of human umbilical vein endothelium with interleukin 1, bacterial endotoxin, or tumor necrosis factor in vitro stimulates production of extracellular matrix, production of procoagulant activity, and enhanced adherence for neutrophils or monocytes.[190–193] Indirect evidence indicates that transient biosynthesis of a factor on the surface of the endothelial cell mediates adherence of neutrophils and monocytes by a CD18-dependent mechanism.[190] Tumor necrosis factor also increases the expression of the CD18 complex on the leukocyte surface.[193] In addition to these stimuli, γ-interferon stimulates an increase in attachment of lymphocytes to endothelial cells.[185,194] The changes in the endothelium leading to the increased adherence of leukocytes are poorly understood. One glycoprotein (intercellular adherence molecule-1) of importance to the attachment of leukocytes to endothelial cells has been partially characterized.[195] It is expressed on surfaces of activated hematopoietic and nonhematopoietic cells and appears to represent the ligand for LFA-1. Expression of this

molecule on vascular endothelium is most intense in inflamed tissues.[196]

Several types of endothelial injury can also enhance adherence of neutrophils to cultured endothelium. Neutrophils adhere more avidly to virally infected endothelial monolayers[197] or to those injured by exposure to excessive oxygen.[198] Proteolysis of fibronectin on the surface of endothelial cells by neutrophil-derived proteases promotes adherence of neutrophils,[199] and these adherent neutrophils may injure the endothelium.[200] Exposure of endothelial receptors for Fc and C3b have been reported following endothelial injury. These receptors could act to localize immune complexes and complement to the vessel wall and thereby increase adhesion by similar receptors present on the neutrophil.[201]

Secretory products of endothelium may also influence adherence. PGI_2, the major arachidonic acid metabolite of large vessel endothelium, has been found to inhibit neutrophil adherence to cultured endothelium.[201] Release of cyclic AMP by endothelial cells stimulated with epinephrine reduces adherence of neutrophils to cultured endothelium in vitro. This could contribute to the decrease in margination and the rise in circulating neutrophil counts observed following epinephrine administration in vivo.[202] Finally, evidence exists that endothelial cells synthesize platelet activating factor, a potent chemotactic factor for neutrophils.[203,204] Such findings support the concept that the endothelium is capable of actively generating mediators that secondarily influence the adhesiveness of neutrophils.

Locomotion into Extravascular Tissues

Electron-microscopic studies have shown that all leukocytes migrate out of vessels by passing between endothelial cells. Recent studies in vitro employing endothelial monolayers in chemotactic chambers indicate that neutrophils insert cytoplasmic processes into intercellular junctions. After penetrating the intercellular junction, the junctions reseal[205,206] and neutrophils appear to hesitate before migrating into the basal lamina.[175,205,207] The mechanisms involved in this emigration process are largely unknown. Specific neutrophil receptors for laminin, an extracellular endothelial matrix protein, may facilitate chemotaxis.[208] Penetration of the vessel wall may require that neutrophils literally digest their way through these cellular junctions as well as the matrix beneath the endothelium by limited release of enzymes. Leukocytes contain cathepsins, elastase, collagenase, and gelatinase, which, if secreted extracellularly, may break down the susceptible connective tissue.[83,107,209] For example, basement membrane destruction or fragmentation is an important feature of immune complex-mediated vascular injury.[207] Clearly, leukocytes are able to invade complex cellular and fibrous tissues. Leukocytes do not demonstrate contact paralysis of locomotion on contact with fibroblasts or endothelial cells in vitro.[210] In fact, contact guidance may contribute to the efficiency of leukocyte locomotion in vivo as suggested by findings of significantly biased cellular locomotion in three-dimensional aligned collagen gels.[211] Intrinsic hydraulic forces rather than adherence requirements may provide a mechanism for locomotion in three-dimensional matrices.[102] Thus, selected tissues may present the cell with a lattice to crawl through, but others, such as the serous lining of cavities or endothelium lining blood vessels, may be more like pain surfaces to which leukocytes must adhere in order to migrate.

CLINICAL DISORDERS OF LEUKOCYTE MOTILITY

General Considerations

The rapid localization of phagocytes to sites of microbial invasion or trauma represents a first-line defense mechanism of particular importance in nonimmune hosts. Quantitative or qualitative aberrations of either the cellular or humoral contributions to these adaptive responses may impair inflammatory defenses and, thus, increase infectious susceptibility. Early animal studies[19] demonstrated a critical 2- to 4-h period after cutaneous invasion by bacterial pathogens during which phagocytic cells must arrive at a site of invasion in order to prevent the establishment of an infectious process. Recurrent bacterial or fungal infections of the skin or mucous membranes are prominent in patients with quantitative deficiencies of peripheral blood leukocytes.[212] Such infections are also evident in patients with qualitative disorders resulting in insufficient accumulation of phagocytes at inflammatory sites, despite normal number of leukocytes in the peripheral blood.[133,213] Among both patient groups, common pathogens such as *Staphylococcus aureus*, *Pseudomonas*, other gram-negative enteric species, or *Candida albicans* account for most infectious complications. Infected tissues in these patients are characteristically gangrenous or necrotic and devoid of pus and contain few granulocytes when examined microscopically. Local inflammatory signs or symptoms in such patients may be minimal though the infection may lead to the destruction of cutaneous, subcutaneous, periodontal, or other submucosal tissues.

Among early reports of clinical disorders typified by susceptibility to recurrent soft tissue infections were patients with abnormalities of leukocyte migration in vitro and/or tissue mobilization in vivo.[6,8,9,214,215] In contrast to observations in patients with chronic granulomatous disease (Chap. 114), studies of granulocytes or monocytes in these patients demonstrated neither abnormalities of microbicidal functions nor granulomatous inflammation in infected tissues. Initially, at least for purposes of comparison of individual patients, a distinct subclassification of disorders of leukocyte motility or chemotaxis seemed justified. However, an explosion of literature followed in which defects of chemotaxis in vitro were associated with a vast array of clinical disorders or conditions. Such reports clearly implied but rarely documented that diminished chemotaxis in vitro was associated with diminished availability or delayed infiltration of phagocytes into inflamed tissues. Correlations between abnormalities of cellular motility in vitro and altered exudation in tissues of human subjects have been infrequent because of the imprecision of skin window techniques.

A reliable interpretation of leukocyte functions in vitro must take into consideration the clinical status of the individual patient, since it is important to determine whether abnormal functions result in increased susceptibility to infection or simply reflect other factors surrounding the patient's condition. Certain pharmacologic agents as well as the nutritional status of the patient may transiently influence selected functions tested in vitro.[25,216] Blood samples obtained for study during the course of infections may contain an increased percentage of immature myeloid cells which function suboptimally.[217] Also, many investigators have reported enhanced, diminished,

or otherwise abnormal motility; phagocytosis; oxidative metabolism; and/or other functions of leukocytes in patients with clinical bacterial infections.[218–221] In most cases these abnormalities are found to be transient, probably reflecting cellular influences of inflammatory mediators[216,222–224] or products of the infecting organisms. Certain bacterial toxins exert significant inhibitory effects on cellular locomotion as well as other functions in vitro.[216] Some such as cholera toxin and certain enterotoxins of *E. coli* exert primarily intracellular effects (e.g., activate adenyl cyclase and elevate intracellular cyclic AMP levels).[225,226] Others preferentially perturb cell membrane properties and include streptolysin O, clostridial toxins (perfringolysin and phospholipase C), a diverse group of staphylococcal toxins (sphingomyelinase C and leukocidin), and proteases (alkaline protease and elastase) elaborated by pathogenic strains of *Pseudomonas aeruginosa*.[227,228] Suggested pathogenic mechanisms related to microbial toxin exposure include alteration of membrane fluidity, inhibition of cytoskeletal protein assemblage, and disruption of membrane receptors for chemotactic factors, complement ligands, or IgG Fc. Finally, certain microbial proteases or other products act directly on humoral mediators of cellular locomotion. For example, elastases elaborated by *P. aeruginosa* cleave C5 (as well as other serum complement proteins), thereby generating complement-derived chemotactic moieties in vitro or in vivo.[227]

The molecular pathogenesis of a limited number of genetic or secondary disorders characterized by defective migration of leukocytes has been defined. Most notable is the heritable deficiency of the adherence proteins of the CD18 complex. The discovery and characterization of this disorder probably best justifies the introduction into this text of a chapter on disorders of motility and adhesiveness in leukocytes. With respect to most of the other disorders to be considered here, a molecular pathogenesis is less clear.

Leukocyte Adhesion Deficiency

Leukocyte adhesion deficiency is a recently recognized autosomal recessive disorder characterized by recurrent bacterial infections, impaired pus formation and wound healing, and a wide spectrum of functional abnormalities in granulocytes, monocytes, and lymphoid cells. The features of this disease result from a deficiency of the CD18 complex of adhesive glycoproteins on the surface of leukocytes. Defective biosynthesis of the β chain common to each glycoprotein represents the fundamental molecular basis of this disease.

In 1980, a patient was reported with severe, widespread, and recurrent infections.[229] As demonstrated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis, lysates of whole neutrophils from this patient lacked a cell surface protein (M_r = 110,000) termed gp110. Subsequently, several similar patients were reported[230–232] with deficiency of surface glycoproteins (M_r = 130,000 to 180,000). Patients' neutrophils and monocytes failed to adhere to a variety of experimental surfaces, failed to undergo chemotaxis in vitro,[132,229,231] and failed to accumulate in artificial inflammatory skin lesions.[231] Receptor-mediated phagocytosis of complement opsonized particles in vitro was also found to be diminished.[132,230] In addition, there were deficiencies of aggregation and antibody-dependent cellular cytotoxicity in vitro.[132] The availability of monoclonal antibodies against the subunits of the CD18 complex of glycoproteins afforded the opportunity to define precisely the molecular deficiency of these patients and other similar patients subsequently identified. Both the α and β subunits of Mac-1, LFA-1, and p150,95 were found to be deficient on these patients' neutrophils, lymphocytes, and monocytes.[132,133,144,147,232–241] A selective deficiency in only one or two of the αβ-complexes has thus far not been reported.[84] Patients' granulocytes and monocytes were also deficient in intracellular pools of αβ complexes as shown by SDS-PAGE of lysates of whole cells.[131,132,147,237] All types of these patients' leukocytes that have been studied were deficient including cultured cytolytic T lymphocytes, mitogen-stimulated T lymphocytes, and transformed B-lymphocyte cell lines.[136,147]

Clinical heterogeneity among patients with leukocyte adherence deficiency is related to quantitative differences in the extent of the molecular deficiency. To date, at least 30 reported patients with recurrent infections and defective adherence, mobility, and phagocytosis have been shown to lack partially or totally the glycoproteins of the CD18 complex.[84,131,132,238–248] Several patients demonstrating similar clinical features and/or functional deficits reported prior to the development of monoclonal antibodies represent presumptive examples of this disease.[246,249–254] One of the earliest patients with abnormal phagocytosis and motility was reported to have actin dysfunction,[246] but later evaluations indicated leukocyte adhesion deficiency.[254] Collectively, this group of patients sharing the same clinical syndrome and the same molecular defect has been referred to in the literature as *Mac-1, LFA-1 deficiency*,[132] *Mo-1 deficiency*,[236] or *deficiency of the CD18 glycoprotein complex*.[255] In the interest of brevity and comprehensiveness, we have suggested the name *leukocyte adhesion deficiency*.[84] Presumptive or confirmed cases reported to date are summarized in Table 113-2.

Clinical and Histopathologic Features of Leukocyte Adhesion Deficiency. Recurrent necrotic and indolent infections of soft tissues primarily involving skin, mucous membranes, and intestinal tract are the clinical hallmarks of this disease (see Fig. 113-2). Superficial infections of body surfaces may invade locally or systemically. Typical small, erythematous, nonpustular skin lesions often progress to large, well-demarcated, ulcerative craters, or pyoderma gangrenosa, which heal slowly or with dysplastic eschars.[84,132] Staphylococcal or gram-negative enteric bacterial organisms may be cultured from such

Table 113-2 Leukocyte Adhesion Deficiency Patients

References	*Confirmed/presumed**	*Female/male*	*Alive*	*Age†*	*Dead*	*Age†*
84, 132, 229–231, 236, 238–246, 250–254, 257	33/26	25/33	28	9 (1–38)	29	1 (0–32)

*Confirmation of the disease requires assessment of leukocytes using monoclonal antibodies specific for CD11/CD18 subunits. Presumed cases exhibited a clinical course and functional abnormalities of leukocytes in vitro consistent with leukocyte adhesion deficiency.

†Age (years) last reported, median (age range).

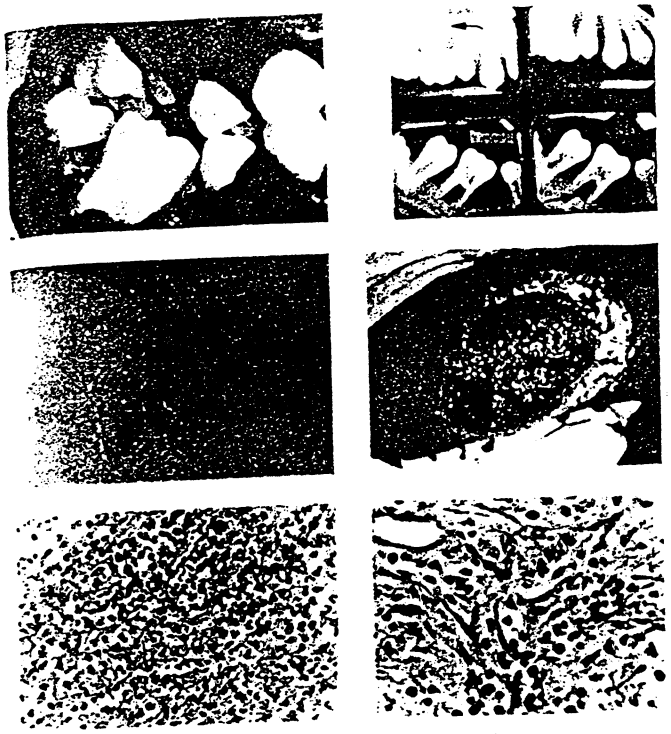

Fig. 113-2 Clinical examples of periodontitis and cutaneous infections in patients with leukocyte adherence deficiency. Severe gingivitis and periodontitis involving the permanent dentition of a 12-year-old patient are shown at the *top left*. Gingivae exhibit acute inflammation, proliferation, recession, and periodontal pocket formation. All remaining teeth exhibit severe mobility. A radiograph of the same area is shown in the *top right* panel demonstrating >60 percent alveolar bone loss around molar teeth. Early erythematous cellulitic lesions with central ulcers from which *Pseudomonas maltophilia* was cultured are shown in the *middle left* panel. An ulcerative necrotic-gangrenous lesion (5 cm diameter) on volar surface of forearm is shown in the *middle right* panel. Sections of infected umbilical cord surgically resected at 18 days of life (hematoxylin and eosin, ×296) are shown at *bottom left* and *right*. A thrombus in an umbilical artery *(left)* contains numerous neutrophils. The inflammatory infiltrate of adjacent connective tissues *(right)* is totally devoid of neutrophils but does contain eosinophils in addition to macrophages, lymphocytes, and plasma cells. *(From D. C. Anderson et al.[132] Used by permission.)*

lesions for up to several weeks despite antimicrobial therapy. Fulminant progression of gas gangrene of soft tissues of a distal extremity in one patient prompted surgical amputation as a lifesaving measure.[231] Septicemia progressing from omphalitis associated with delayed umbilical cord severance has been observed in several families.[84,132,249,250] Perirectal abscess or cellulitis leading to peritonitis and/or septicemia has been reported in multiple patients, and facial or deep neck cellulitis has been observed to progress from ulcerative mucous membrane lesions of the oral cavity.[132,230,231] Recurrent invasive candidal esophagitis, erosive gastritis, acute appendicitis, and necrotizing enterocolitis have been reported in multiple patients.[84] Recurrent otitis media occurs commonly, and progression to mastoiditis and facial nerve paralysis has been reported. Other common respiratory infections include severe bacterial (pseudomonal) laryngotracheitis, recurrent pneumonitis, and sinusitis.[84] Severe gingivitis and/or periodontitis is a major feature among all patients who survive infancy. Acute gingivitis has appeared in all cases with eruption of the primary dentition. Subsequently, these patients develop characteristic features of progressive generalized prepubescent periodontitis, including gingival proliferation, defective recession, mobility, pathologic migration, and advanced alveolar bone loss associated with periodontal pocket formation and partial or total loss of both the deciduous and permanent dentitions.[132,248]

The recurrent infections observed in affected patients appear to reflect a profound impairment of leukocyte mobilization into extravascular inflammatory sites. Skin windows as well as biopsies of infected tissues demonstrate inflammatory infiltrates totally devoid of neutrophils.[132,231,245] This histopathologic feature is particularly striking considering that marked peripheral blood leukocytosis (five- to twentyfold normal values) is a constant feature of this disorder. Transfusions of leukocytes result in the appearance of donor neutrophils and monocytes in skin windows and in skin chambers.[231] Impaired healing of traumatic or surgical wounds observed in several patients represents a clinical feature not generally observed in patients with neutropenia or dysfunctional neutrophils. Unusual paper-thin or dysplastic cutaneous scars have been found in some patients.[132,239] This may reflect the lack of monocyte infiltration and the lack of inflammatory contributions to healing such as the elaboration of angiogenesis factors.[132] The wide spectrum of gram-positive or gram-negative bacterial and fungal infectious microorganisms[84] is also characteristic of patients with primary neutropenia syndromes. These clinical models also demonstrate insufficient tissue leukocyte infiltration. However, deep-seated granulomatous infections typical of chronic granulomatous disease and other examples of oxidative or nonoxidative intracellular killing deficits have not been observed.

Some evidence suggests that patients with leukocyte adhesion deficiency have an increased susceptibility to viral infection. Most patients have demonstrated normal and self-limiting courses of varicella or other viral respiratory infections, and 5 of 10 patients in one report[132] demonstrated no untoward reactions to live viral vaccine administration. However, one patient died of an overwhelming infection with picornavirus involving oral pharynx, glottis, trachea, and lungs, and three patients of the same series had one or more episodes of aseptic (presumably viral) meningitis.[132]

The severity of clinical infectious complications among these patients appears to be directly related to the degree of glycoprotein deficiency. Two phenotypes, designated severe and moderate deficiency, have been identified (Table 113-3).[84,132] As measured by immunofluorescence flow cytometry

Table 113-3 Clinical Features of Mac-1, LFA-1, p150,95 Deficiency Syndrome in Texas Patients[84,132]

Clinical features	*Severe**	*Moderate†*
Delayed umbilical cord severance	3/4	0/6
Persistent granulocytosis (15,000–161,000/mm^3)	4/4	6/6
Recurrent infections:		
Cutaneous abscess or cellulitis	4/4	6/6
Perirectal cellulitis with sepsis	4/4	0/6
Stomatitis and facial cellulitis	4/4	3/6
Gingivitis and periodontitis	4/4	6/6
Pneumonitis necrotizing enterocolitis, peritonitis	4/4	2/6
	2/4	0/6
Impaired wound healing	3/4	2/6
Parental consanguinity	2/4	3/6
Age range (years)	1 to 6	11 to 38

*Leukocytes from these four patients had less than 0.3 percent normal amounts of Mac-1 on their surfaces.
†Leukocytes from these six patients had 2.5 to 6 percent normal amounts of Mac-1 on their surfaces.

and verified by radioimmunoassay and immunoprecipitation techniques, four severely deficient patients had essentially undetectable expression (<0.3 percent of normal amounts) of all three αβ complexes on their neutrophils. Six moderately deficient patients expressed 2.5 to 6 percent of all three αβ complexes. Patients with severe deficiency have either died in infancy or demonstrated a suceptibility to severe, life-threatening systemic infections (peritonitis, septicemia, pneumonitis, aseptic meningitis). In contrast, among the six patients with moderate deficiency (mean age 21 years, range 9 to 38 years) life-threatening infections have been infrequently observed despite a relatively prolonged survival.[132] Patients within a kindred demonstrate similar survival periods. For example, in one study, three patients with moderate disease died at ages 22, 19, and 32 years.[256] In other studies of patients with severe disease, five infants died in their first year and one died at 3 years of age.[250,252,253] In some moderately affected patients, skin lesions may disappear after the first few years of life, recurring only with occasional infections. Severe gingivitis is always observed in these patients and may be the presenting symptom.[248] Delayed umbilical cord separation occurs more frequently in patients with the severe phenotype, but it is not universally found.

Functional Abnormalities in Patients with Leukocyte Adhesion Deficiency. Some of the heterogeneity in functional abnormalities found among individual patients or kindreds may reflect methodologic differences among reporting laboratories.[84] However, abnormalities of adherence to substrates and adhesion-dependent functions including chemotaxis and aggregation have been observed among all patients studied.[132,133,229] Chemotaxis appears to be affected because it requires adhesion.[102,132] CR3-dependent binding and phagocytosis of iC3b-opsonized particles are deficient, in agreement with the identity of the CR3 with Mac-1.[128] In addition, since particles opsonized with iC3b are phagocytosed poorly, they fail to trigger the respiratory burst.[131,132,230,242,249,256,257] Abnormalities of antibody-dependent cellular cytotoxicity have also been observed in several patients.[123,132,133] In contrast, adherence-independent cellular functions including f-Met-Leu-Phe receptor-ligand binding and oxidative metabolism or degranulation mediated by soluble stimuli are generally normal.[131,132,231,238,239] Intracellular microbicidal activity (e.g., the ability to kill *S. aureus*) in most reported patients is relatively normal.[131,132,231,256] This indicates that receptors other than CR3 (e.g., Fcγ or CR1) are sufficient to promote a normal level of phagocytosis and intracellular killing in most instances.[117,131,132,147] Overall, more profound functional abnormalities have been observed among severely deficient as compared with moderately deficient patients.[84,132]

The predominance of recurrent bacterial (as opposed to viral or fungal) infections in patients with leukocyte adhesion deficiency implies that the functions of neutrophils or monocytes are more profoundly affected than those of lymphocytes. However, deficits of the LFA-1-dependent functions of lymphocytes have been observed in many patients. Furthermore, in cases where LFA-1-dependent functions are nearly normal, they are inhibited by much lower concentrations of monoclonal antibodies to LFA-1 than for normal cells. T lymphocyte-mediated killing, proliferative responses, natural killing, and antibody-dependent killing by patients' lymphocytes are deficient compared with that in adult controls.[123,133,136,238,245,249,251,258] In primary mixed lymphocyte culture, lymphocytes in several studies have demonstrated profoundly diminished cytotoxic and proliferative responses and interferon production.[136,235,238,251] However, after further stimulation, these responses increase to nearly normal levels.[136] This may be due to compensatory mechanisms, perhaps involving an increase in the affinity of the T-lymphocyte antigen receptor, and may account for the relatively normal functions of B and T lymphocytes observed in most cases. Delayed cutaneous hypersensitivity reactions are normal in most patients tested, and most individuals demonstrate normal specific antibody synthesis.[132,244] However, T-lymphocyte-dependent antibody responses in vivo (for example, to repeated vaccination with tetanus, diphtheria toxoids, and polio virus) are impaired, and antibody production in vivo or in vitro in response to influenza virus was found to be abnormal in one patient.[259] Thus, responses of lymphocytes in vivo may be found deficient in only some of the patients whose β-subunit mutation is particularly deleterious to the expression of LFA-1.

Inheritance of Leukocyte Adhesion Deficiency. Several lines of evidence indicate that leukocyte adhesion deficiency is transmitted as an autosomal recessive disorder in most, if not all, cases. Individuals who are clinically unaffected but appear to be heterozygotes have been identified by expression of approximately 50 percent of normal amounts of Mac-1α/β subunits on the surface of their neutrophils following stimulation with chemotactic factors such as f-Met-Leu-Phe.[131,132] In three families, all the clinically unaffected mothers and fathers and some of the sibs were found to be heterozygotes.[132] In one family spanning three generations, an affected son was born to heterozygous parents. The affected son married a heterozygote, and the couple bore an affected son and daughter and two heterozygous daughters. These findings, together with the overall equal numbers of male and female patients recognized worldwide (Table 113-2)[132,241,242,250–252,256] and a frequent history of consanguineous marriages,[132,238,244,249,253,257] strongly suggests that leukocyte adhesion deficiency is inherited as a recessive trait on an autosomal chromosome. In one family, X-linked inheritance was suggested,[230,236] but there is no definitive evidence for an X-linked form of leukocyte adhesion deficiency.

The molecular basis of leukocyte adhesion deficiency has been studied at the protein, mRNA, and DNA levels. Biosynthesis has been studied using Epstein-Barr virus transformed B lymphocyte and mitogen-stimulated T-lymphocyte cell lines, and healthy individuals synthesize the LFA-1α subunit and the common β subunit and express the LFA-1 αβ complex on the cell surface. Cell lines from patients synthesize an apparently normal LFA-1α subunit precursor, but this precursor does not undergo carbohydrate processing, does not associate in an αβ complex, and is not expressed on the cell surface (Fig. 113-3). The LFA-1α subunit is apparently degraded in the absence of the β subunit.[147]

In hybrids of human and mouse lymphocytes, human LFA-1α and β subunits from healthy controls associate with mouse LFA-1α and β subunits to form interspecies hybrid αβ complexes.[260] In hybrids of patient and mouse lymphocytes, the α but not the β subunit was rescued by the formation of interspecies complexes that were expressed on the hybrid cell surfaces. These findings show that the LFA-1α subunit in genetically deficient cells is competent for surface expression in the presence of an appropriate mouse β subunit, suggesting indirectly that the genetic lesion affects the β subunit.

Recently, the β subunit polypeptide has been purified to homogeneity and the β subunit cDNA has been cloned and

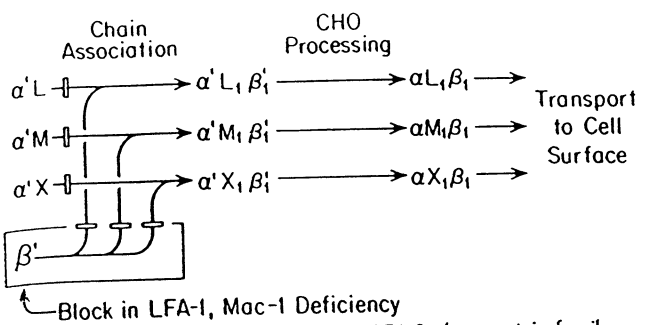

Fig. 113-3 Biosynthesis of the Mac-1, LFA-1 glycoprotein family. The biosynthetic pathway in normal cells is as described in Ref. 124. The evidence for a primary block in β-subunit synthesis, a secondary block in αL biosynthesis due to a lack of β-subunit association, and hypothetically similar blocks in αM and αX biosynthesis is discussed in the text. Precursors are indicated as α' and β', while mature subunits are indicated as α and β. *(From T. A. Springer et al.[147] Used by permission.)*

sequenced.[145,261] Additionally, the development of rabbit antihuman β-subunit antibodies allows immunoprecipitation of β-subunit precursors in both healthy and deficient cell lines and permits their examination in SDS-PAGE.[262,263] Normal quantities of the β-subunit precursor and β-subunit mRNA were found in four unrelated patients.[263] However, the β-subunit precursors, as previously shown for LFA-1α subunit precursors,[147,264] did not undergo normal carbohydrate processing. In a study of six unrelated patients and four related patients and other members of their kindred the following five distinct variations in the β subunit were identified among the different mutant alleles:[262] (1) The subunit was undetectable. (2) The quantities of β-subunit mRNA and protein precursor were low. (3) An aberrantly large β-subunit precursor likely due to an extra glycosylation site was found. (4) An aberrantly small β-subunit precursor due to a polypeptide chain defect was found. (5) No gross abnormality in the β-subunit precursor was found. In studies of one kindred (see Fig. 113-4), including four related patients of the moderate phenotype, a β precursor of identically abnormal small size was identified in each case. Of 10 relatives within this family, nine have been typed as heterozygous carriers and one as a noncarrier.[132] All nine heterozygotes show both a normal and an abnormally small β precursor; the noncarrier shows only the normal β precursor. These studies provided conclusive evidence that the defect is in the gene for the β subunit.[262]

Differences in the β-subunit precursor between unrelated patients suggest distinct mutations in the β-subunit gene. This means that while the moderate and severe phenotypes are useful in a broad sense for categorizing patients, some heterogeneity in the severity or spectrum of clinical symptoms within each category is to be expected. While there is no obvious molecular explanation for the moderate and severe phenotypes, it is possible that mutant β subunits vary in their ability to complex with the α subunits and that this determines the amount of the αβ complex expressed on the cell surface. The β subunit and hence the genetic lesion has been mapped to chromosome 21 in somatic cell hybrids and by *in situ* hybridization (Ref. 260 and unpublished). This is in agreement with autosomal inheritance.

Therapeutic Considerations for Leukocyte Adhesion Deficiency. Bone marrow transplantation with successful engraftment rendered unnecessary any further treatment in two patients.[249] In two other patients, successful engraftment was achieved, but recovery did not occur either because of graft-

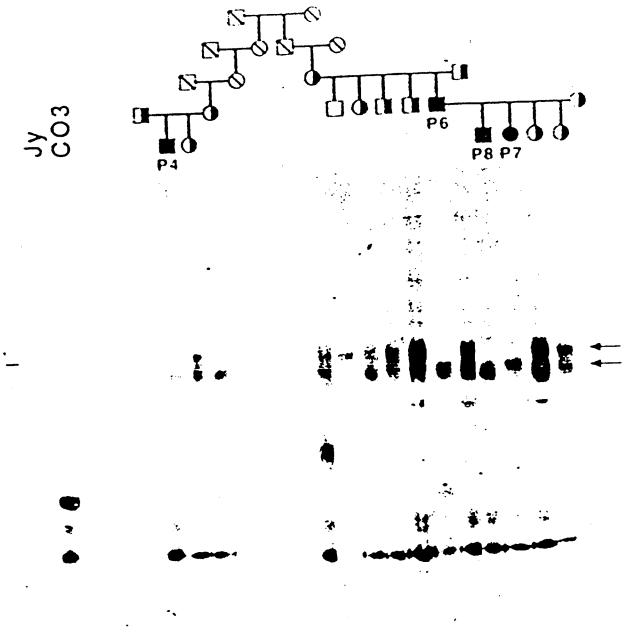

Fig. 113-4 Inheritance of an aberrantly small β precursor (β') within a moderate kindred with leukocyte adhesion deficiency. Homozygous deficient patients (P4, 6, 7, and 8) (closed symbols), heterozygotes (half-filled symbols), or homozygous noncarriers (open symbols) demonstrate only abnormal β, abnormal and normal β precursor, or only normal β precursor, respectively (see Ref. 132). EBV-transformed cells from a control cell line (JY) and from patients with leukocyte adhesion deficiency and their kindred were pulse-labeled with [^{35}S]methionine. The β-subunit precursors were immunoprecipitated with rabbit anti-β-subunit serum and protein A-sepharose. Samples were subjected to SDS-PAGE and autoradiography with fluorography. The normal β precursor and the aberrantly small β precursor are indicated by arrows. *(From T. K. Kishimoto et al.[262] Used by permission.)*

versus-host disease or infectious complications. Transplantation is recommended for severely deficient patients because of the high incidence of death before age 2. Moderately deficient patients live longer but may also be susceptible to life-threatening infections. The occurrence of deaths at ages 19, 22, and 32 (Table 113-2) and the absence of any known patients older than 40 years shows that survival through adolescence is no guarantee of a long life. Therapeutic guidelines for management of moderately deficient patients are not well defined.

HLA-mismatched bone marrow transplantation has proven successful in many different diseases recently. This has been made possible in part by depletion of donor T cells thus preventing graft-versus-host disease. However, the incidence of rejection of the graft by the recipient has become more troublesome. It has been observed that LFA-1 deficient patients, none of whom mounted allogeneic mixed lymphocyte responses, did not reject grafts.[247] Since graft rejection can be mediated by both T and non-T cells, monoclonal antibodies against LFA-1 inhibit both T and natural killer immune functions in vitro,[134] and LFA-1 is not on hematopoietic stem cells,[265] graft recipients were treated with 0.1 mg/kg anti-LFA-1α subunit monoclonal antibodies for 3 days before and 5 days after transplantation. Recipients had a variety of inherited diseases such as Wiskott-Aldrich syndrome and osteopetrosis, and all received HLA-mismatched transplants. The use of monoclonal antibodies against LFA-1 resulted in seven of seven successful engraftments, a clear improvement over previous experience.

The fact that the genetic lesion occurs in the common β

subunit of the CD18 glycoprotein family opens the possibility that introduction of a normal β-subunit gene into hematopoietic cells should cure the disease. Since the mouse β subunit has been shown to complex with and rescue the surface expression of the human LFA-1α subunit in hybrids of patient and mouse lymphocytes,[260] it seems likely that introduction of a normal human β-subunit into patients' cells would result in expression of functional αβ complexes. Efforts are now being directed toward introducing the cloned β-subunit gene[145] into bone marrow cells using retroviral vectors. Thus, the feasibility of somatic gene therapy for this disease is now being explored.

Clinical Conditions Characterized by Abnormally Elevated Expression of CD18 Glycoproteins

Considering the many contributions of CD18 glycoproteins to cellular adherence and the importance of adherence in the inflammatory response, it is not surprising that abnormalities in this protein complex have been identified in other diseases. These include intrinsic cellular defects such as occur in neonatal leukocytes[266,267] as well as abnormalities secondary to extracellular inflammatory mediators.

High serum or tissue levels of biologically active complement fragments may pathologically elevate the adherence of circulating leukocytes and thereby promote leukocyte aggregation, sequestration, and an associated systemic leukopenia.[169] When infused into experimental animals, complement derived chemotactic factors activate peripheral blood phagocytes, which in turn, avidly adhere to one another or to endothelium of pulmonary vasculature.[268] These metabolically activated cells may obstruct blood flow as well as damage endothelial and pulmonary tissues through oxygen radical generation and lysosomal enzyme release.[167,269] This sequence of events is thought to contribute to acute respiratory symptoms, multiple organ injury, and leukopenia associated with gram-negative septicemia, endotoxemia, and other complement activation states, including hemodialysis, filtration leukapheresis, cardiopulmonary bypass, pancreatitis, and trauma.[101,167,169,268,270–272] Increasing evidence suggests that the CD18 glycoproteins play an important role in these events.

Pulmonary sequestration of leukocytes in patients undergoing hemodialysis has been correlated with increases in Mac-1 on circulating leukocytes.[273] In eight patients undergoing maintenance hemodialysis, there was a fivefold increase in the mean expression of Mac-1 on neutrophils within 15 min after the start of dialysis with a new cuprophane membrane. The peak increase coincided with the maximum drop in neutrophil count and with the peak rise in the plasma levels of the complement activation products $C5a_{desarg}$ and $C3a_{desarg}$. $C5a_{desarg}$ induced a comparable increase in Mac-1 expression on normal neutrophils in vitro at concentrations similar to those measured in vivo. Chemotactic peptides induced aggregation of normal neutrophils (a reflection of increased cell-cell adhesiveness), and the aggregation was specifically and totally blocked by mouse monoclonal antibodies to Mac-1 in vitro. Thus, it appears that increased expression of Mac-1 during complement activation by cuprophane membranes contributes to the onset of leukoaggregation and granulocytopenia in the hemodialysis model.

In other studies using isolated perfused rodent lungs,[274] monoclonal antibodies against Mac-1 attenuated sequestration, superoxide generation, lysozyme release, and lung injury caused by infusion of human neutrophils stimulated by phorbol myristate acetate. In addition, lung injury was not observed in this experimental model following infusion of human neutrophils from a patient with leukocyte adhesion deficiency.

Another clinical syndrome with associated acute respiratory symptoms appears to involve the CD18 glycoproteins. Recent evidence indicates that the fungicidal polyene antibiotic amphotericin B promotes elevated expression of CR-1 and CR-3 and aggregation of human neutrophils in vitro.[275] This finding suggests that the acute pulmonary symptoms observed in hematologic patients simultaneously receiving amphotericin B and leukocyte transfusions are related to a CR-3-dependent pathogenic mechanism.[276]

Clinical Conditions Associated with Undefined Abnormalities of Cellular Adherence

Patients with poorly controlled diabetes mellitus exhibit impaired neutrophil adherence to nylon fibers or glass wool, reduced chemotaxis in vitro, and reduced leukocyte mobilization in vivo.[277] Improved metabolic control appears to correct the adhesive abnormalities.[278,279] Though these deficits are thought to contribute to infectious susceptibility in affected individuals, studies of neutrophil adherence under conditions of chemotactic activation or studies of leukocyte-endothelial interactions have not been reported. Thus, neither the extent of the abnormalities nor the mechanisms have been characterized.

Adhesive and migratory properties of phagocytic cells are influenced by a number of pharmacologic agents including β-adrenergic agonists, steroids, and nonsteroidal anti-inflammatory agents. The peripheral blood leukocytosis occurring in subjects receiving epinephrine is due to reduced adherence of neutrophils mediated through β receptors on endothelial cells.[202] Endothelial cells respond to catecholamine exposure in vitro by raising concentrations of cyclic AMP, a metabolic intermediate known to diminish leukocyte adherence.[280] Thus, cyclic nucleotides of endothelial origin appear to regulate physiological margination-demargination via secondary effects on adherence properties of circulating leukocytes.

Disorders Secondary to Abnormal Secretory Functions

Neutrophils contain multiple subpopulations of granules.[281] Azurophil or primary granules appear early in neutrophil development and contain lysosomal enzymes, including lysozyme and myeloperoxidase. Specific or secondary granules develop later. Though they lack myeloperoxidase and other hydrolases, specific granules are capable of extracellular release of a number of substances such as lactoferrin that may regulate inflammation.[83,282] The first example of a deficiency of specific granules was recognized in 1972.[283] Other cases have been subsequently reported by several laboratories.[115–117,284–289] One patient[283] appears to have had an acquired deficiency (associated with a myeloproliferative syndrome), while all others appeared to have genetically determined disease. Each has demonstrated susceptibility to recurrent and severe infections of the skin, mucous membranes, and lung, most commonly due to *S. aureus*, *P. aeruginosa*, other enteric pathogens, and *C. albicans*. Infections may progress from superficial sites; otitis media with associated mastoiditis was reported in one patient, and lung abscess formation due to *S.*

aureus followed the onset of pneumonia in another individual. The occurrence of necrotic oral lesions due to invasion by *E. coli* and species of *Pseudomonas* and *Klebsiella* was reported in another individual, but severe neutropenia recognized in that patient may have accounted for the development of these mucous membrane lesions.[285] Another patient[116] with severe scalp infections due to *Proteus mirabilia* and *S. aureus* required prolonged intravenous antibiotic therapy in addition to surgical debridement. Detailed descriptions of the histopathology of infected tissues in all patients are not reported, but skin window studies have demonstrated diminished pus formation in tissues of some individual who were not neutropenic.[115,116]

Neutrophils from each patient studied have demonstrated morphologic abnormalities, including a severe or total deficiency of specific granules and a variety of nuclear abnormalities including bilobed or multilobed nuclei or nuclear blebs, clefts, or pockets. Diminished or absent neutrophil lactoferrin content has been confirmed in only three cases, and the membrane marker alkaline phosphatase has been shown to be diminished or absent in neutrophils of all but one reported case. Total cellular content and/or release of the secondary granule markers (lactoferrin, B_{12} transport protein, cytochrome b, and lysozyme) have been shown to be diminished when assessed in selected patients, although levels of primary granule constituents (myeloperoxidase, β-glucuronidase) are generally normal.

Among recognized cases, somewhat heterogenous abnormalities in cellular functions have been observed. Chemotaxis and intracellular microbicidal activity represent the most consistently reported functional deficits. The basis of impaired leukocyte locomotion in vitro or diminished accumulation in skin windows in vivo is uncertain. However, in studies of two patients[115–117] defective cellular migration appeared to be functionally related to abnormalities of adherence. In one patient there was diminished adherence to nylon fibers and endothelial cells and impaired aggregation in response to f-Met-Leu-Phe.[115] In response to f-Met-Leu-Phe, another patient's neutrophils failed to enhance CR-3 expression although upregulation of CR-1 was normal. Immunoprecipitation experiments employing anti-CR-3 or anti-CR-1 and fractionated normal neutrophils showed that CR-3 was associated with plasma membrane and specific granule enriched fractions, while CR-1 was associated only with plasma membrane. Such findings are consistent with the hypothesis that CR-3 but not CR-1 is associated functionally and anatomically with the specific granules of neutrophils. Supporting data from two laboratories[117,290] demonstrate abnormal expression of Mac-1 on neutrophils deficient in specific granules.

Deficiency of specific granules is suggested by a history of recurrent cutaneous, subcutaneous, mucous membrane, or pulmonary infections due to *S. aureus*, virulent gram-negative enteric bacteria, or species of *Candida*. Findings of abnormal morphology and abnormally weak cytochemical reactions for alkaline phosphatase are highly suggestive of this disorder. Cytochemical and ultrastructural studies to confirm diminished numbers or abnormal morphology of specific granules and their specific constituents will establish a diagnosis. While most examples of specific granule deficiency recognized to date are probably genetic in origin, the mode of transmission of this disorder is uncertain. A prognosis is not well defined, but most individuals have survived the pediatric age group with antimicrobial and supportive therapy.

The molecular basis of the complex alterations of cellular functions in the syndromes associated with deficiency of specific granules remains undefined. Data presently available generally indicate that one or more specific granule constituents are required for or participate in neutrophil locomotion or oxidative metabolism in vitro. Considering the limited population of patients studied as well as the complex nature of functional or biochemical abnormalities, an interpretation of the precise pathogenic determinants of infectious suceptibility in these syndromes is not possible.

Functional Abnormalities in Neonatal Neutrophils

Since specific immunity is severely limited in the immediate postpartum period, the inflammatory functions of phagocytic cells are especially important for host defense against microbial invasion.[291] Both quantitative and qualitative abnormalities of phagocytic cells contribute to the enhanced infectious suceptibility of neonates. Neutropenia is commonly observed in systemically infected neonates, and studies in neonatal animals indicate that exhaustion of a limited reserve pool of bone marrow granulocytes contributes to a depletion of circulating or marginating pools when tissue demand is increased.[292] Among the most consistently observed functional abnormalities thought to contribute to impaired inflammation in neonates are those related to the motility of leukocytes.[24] As shown with skin windows, inflammatory responses in newborns differ from those in older children and adults in two respects: (1) the shift from the early granulocyte predominance to a predominance of mononuclear cells is slower and less pronounced, and (2) a marked eosinophilia is observed in some infants 2 to 21 days of age.[293,294] Strikingly diminished leukocyte mobilization in neonatal rats inoculated intraperitoneally with bacteria or chemotactic agents has been demonstrated.[295]

Neonatal neutrophils exhibit impaired chemotactic response to numerous chemotactic factors including those released by growing *S. aureus* and *E. coli* (e.g., f-Met-peptides) and those generated in plasma by antigen-antibody complexes (e.g., C5a).[24,296] Visual assays demonstrate that in addition to depressed migration, neonatal cells are significantly impaired in their ability to orient toward a gradient of chemotactic factors.[24,297] Depressed chemotaxis has been found in healthy neonates 1 to 5 days of age.[298,299] In addition, there is diminished generation of chemotactic activity (chemotaxigenesis) by virulent type III group B streptococci in neonatal sera related directly to diminished levels of both type-specific anticapsular antibody and serum complement activity.[300] Thus, impaired generation of chemotactic stimuli as well as abnormal cellular response appear to account for diminished inflammatory responses observed in even healthy term neonates.

Evidence exists that impaired chemotaxis of neonatal neutrophils is functionally linked to abnormalities in cellular adherence.[24,297,301,302] The modulation of adherence induced by chemotactic stimulation of adult neutrophils occurs to a very limited extent in neonatal neutrophils. Adherence is poorly enhanced by exposure of cells to C5a, f-Met-Leu-Phe, or bacterial chemotactic factors, and the movement of adhesion sites from front to tail of cells polarized by chemotactic stimulation is greatly reduced.[24,300] There is also impaired lateral mobility of lectin (concanavalin A) receptors on neonatal granulocyte surfaces most likely reflecting abnormal membrane fluidity.[217,303–307] Secretory abnormalities may also contribute to impaired adherence.[266,308] The following experimental observations support this possibility. Baseline expression of Mac-1 and receptors for f-Met-peptides on the surface of neonatal

neutrophils has been found to be normal, but upregulation of these proteins following stimulation with chemotactic factors is significantly reduced compared to that in adult cells.[266] Ultrastructural assessments of neonatal neutrophils have demonstrated significantly less peroxidase-negative granule loss following chemotactic stimulation than adult cells. In addition, the release of lactoferrin, a marker of specific granules, following stimulation of neonatal neutrophils with secretagogues such as phorbol esters, is abnormal.[266,308] These studies suggest that abnormal expression of multiple surface determinants derived from peroxidase-negative (specific) granules or other intracellular pools may contribute to deficient chemotaxis and other inflammatory functions of neonatal granulocytes.[266]

Because multiple host defense mechanisms are defective or developmentally delayed in human neonates, a precise cause-and-effect relationship between impaired cellular migration and the occurrence of infectious complications cannot be established. However, neonates are particularly susceptible to the development of cutaneous inflammatory lesions or abscesses at sites of local trauma (for example, circumcision wounds, umbilicus, intertriginous areas, or sites of electrode-monitoring devices). Further, microorganisms such as *S. aureus*, gram-negative rods, and species of *Candida* represent the most common agents infecting cutaneous or mucous membrane lesions in human neonates. The propensity for systemic invasion and the development of neonatal septicemia by endogenous respiratory or gastrointestinal flora may also be related to insufficient infiltration of granulocytes or monocytes into submucosal tissues.[274,295,309]

Chediak-Higashi Syndrome

The Chediak-Higashi syndrome is an autosomal recessive disorder of mink, cattle, beige mice, and humans. This condition is characterized clinically by partial oculocutaneous albinism, the presence of giant lysosomal granules in all granular cell types, susceptibility to bacterial infection, variable occurrence of neutropenia and thrombocytopenia, and an accelerated lymphomalike proliferative phase generally occurring in the first decade of life.[310–312] Infectious complications are attributable to neutropenia as well as functional deficits of neutrophils, monocytes, and/or natural killer (NK) cells. A comprehensive review in 1972[310] documented the significance of infectious morbidity and mortality in this syndrome. Among 56 cases reviewed, 33 individuals died prior to 10 years of age; among 27 cases for whom a cause of death could be determined, infections represented the sole cause in 17 and a contributing factor in 9 additional cases. Pulmonary, cutaneous, subcutaneous, and upper respiratory infections were most commonly observed. *S. aureus* accounted for approximately 70 percent of all infections for which an etiologic agent was determined; group A *Streptococcus*, gram-negative enteric organisms (*Klebsiella, Pseudomonas, Proteus, Shigella* species), and *Aspergillus*, or species of *Candida* represented occasional etiologic agents.

Neutrophils, monocytes, and lymphocytes from these patients demonstrate large intracellular inclusions or granules, which represent the pathologic hallmark of the disease. Although they are most easily demonstrated in leukocytes, they are also present in renal tubular epithelium, gastric mucosa, pancreas, thyroid, neural tissue, and melanocytes.[310] In neutrophils, inclusions contain azurophilic granule markers (myeloperoxidase and acid phosphatase) and have been assumed to represent abnormal azurophilic granules. However, these abnormal granules contain both azurophilic and specific granule markers.[313] Normal appearing specific granules are present, but normal azurophilic granules have not been seen. Analysis of bone marrow samples from patients with Chediak-Higashi syndrome suggest that abnormal granules are formed during granulocyte maturation by the progressive aggregation and fusion of azurophilic and specific granules. Such findings are consistent with a proposed membrane abnormality.[313,314]

Several functional abnormalities of neutrophils, monocytes, and natural killer cells of these patients have been identified. Neutrophils demonstrate delayed and diminished intracellular killing of both gram-positive and gram-negative bacterial organisms, despite a normal capacity to ingest these organisms and a normal or elevated oxidative burst.[310,315] Microbicidal abnormalities are attributed to impaired postphagocytic phagolysosomal fusion.[315] A rather selective impairment of the functions of natural killer cells (as opposed to other lymphocyte functions) has been reported.[316–319] Dysfunction of the natural killer cell system may account for the ultimate development of an aggressive lymphoproliferative syndrome in most patients.

Chemotaxis in vitro and leukocyte mobilization in vivo using the skin window technique are abnormal in patients with Chediak-Higashi syndrome.[311] In micropore filter assays employing a 5-μm pore size, the chemotactic activity of the patient's neutrophils was approximately 41.2 percent of that of controls. When the filter size was decreased to 1.2 μm, chemotaxis of the patient's cells was only 9.5 percent of that of the controls. These findings suggest that granular structures may mechanically impair migration through such small pores. A relationship of impaired leukocyte function to an underlying disorder of microtubule function and/or cyclic nucleotide metabolism has been suggested but not proven.[320–326]

A diagnosis of Chediak-Higashi syndrome can be ascertained by identifying characteristic phenotypic features of the disorder in addition to characteristic large cytoplasmic inclusions in all granular cells, including peripheral blood granulocytes. Giant melanosomes can be demonstrated from hair of patients. Neutropenia and thrombocytopenia are most characteristic during the accelerated phase of disease. Common abnormalities observed on examination of bone marrow aspirates include hypercellularity with extensive vacuolization and inclusions in myeloid precursors. Elevated serum lysozyme levels probably reflect intramedullary granulocyte destruction.[310] The accelerated phase of Chediak-Higashi syndrome is characterized by widespread tissue infiltrates of lymphoid and histiocytic cells usually without malignant histologic characteristics.[319,327] Splenomegaly and associated hypersplenism contribute to observed anemia and thrombocytopenia and may also contribute to the occurrence of neutropenia. Although viral agents and/or immunologic mechanisms may contribute to the pathogenesis of the accelerated phase, the precise mechanisms are undefined.

Most patients with Chediak-Higashi syndrome succumb to infectious or infiltrative complications within the first decade of life. Successful bone marrow transplantation with reversal of the defect in natural killer activity has been reported in one case.[328] Definitive preventive or therapeutic strategies await definition of its molecular pathogenesis.

Type 1b Glycogen Storage Disease

The association of neutropenia, impaired neutrophil migration, and recurrent infection in type 1b glycogen storage disease was first reported in 1980.[329] Most clinical features of type 1b glycogen storage disease are similar to those of type 1a glycogen storage disease, including hepatomegaly, fasting hypoglycemia, lactic acidosis, short stature, hyperlipidemia, and the occurrence of hepatomas with potential for malignant degeneration (see Chap. 12). Patients with type 1a glycogen storage disease demonstrate a deficiency of glucose-6-phosphatase activity in liver, kidney, and intestine. In contrast, type 1b glycogen storage disease patients demonstrate normal glucose-6-phosphatase activity.

A review of the clinical and laboratory features of 21 patients with type 1b glycogen storage disease[330] indicated that most suffered from a variety of moderate to severe bacterial infections including pneumonitis, recurrent otitis media, subcutaneous abscesses, generalized pyoderma, cellulitis, wound infections, and osteomyelitis, most commonly secondary to *S. aureus*. Most patients exhibited chronic neutropenia, which, in some patients, was associated with demonstrable serum inhibitors of myeloid stem cell proliferation, abnormalities of myeloid maturation, and/or decreased peripheral marginating pools. Functional abnormalities including diminished random or directed migration of neutrophils in vitro was documented in 8 of 11 patients tested, and deficient chemotactic modulation of adherence by chemotactic factors was observed in two patients.[26] In contrast, microbicidal activity of neutrophils and phagocytosis-associated oxidative metabolic activity have been shown to be normal in most patients with type 1b glycogen storage disease.[330]

The biochemical basis for quantitative or qualitative abnormalities of neutrophils or mononuclear leukocytes is uncertain. However, glucose-6-phosphatase activity in liver homogenates from patients with type 1b glycogen storage disease was normal only when assayed in the presence of detergents (e.g., triton X-100).[329,331] The high latency (90 percent) indicates that detection of this activity is dependent on detergent to disrupt microsomes. Further studies in one patient[332] identified a defect of glucose-6-phosphatase translocase, one of three integral membrane components of the hepatic microsomal glucose-6-phosphatase system (see Chap. 12). A physiological role of glucose-6-phosphate transport in neutrophils has not been defined, and thus a causal relationship between aberrant glucose-6-phosphate transport and impaired neutrophil migration cannot yet be established.

Mannosidosis

Mannosidosis is a lysosomal storage disease characterized clinically by psychomotor retardation, facial dysmorphology similar to that of Hurler syndrome, dysostosis multiplex, hepatosplenomegaly, hearing loss, and recurrent soft tissue infections (see Chap. 63). This autosomal recessive disease is due to a deficiency of acidic α-mannosidase activity resulting in mannose-rich oligosaccharide accumulation in lysosomes of circulating leukocytes and in neural and visceral tissues. A defect of neutrophil chemotaxis and phagocytosis in neutrophils and diminished lymphocyte transformation were described in one child with systemic mannosidosis.[333] It was suggested that these functional defects result from abnormal mannose catabolism and that partially degraded oligosaccharides, glycopeptides, glycoproteins, and terminal αD-mannose residues may bind to leukocyte plasma membranes as well as accumulate in lysosomal granules. In a review of 17 cases, 13 patients experienced significant or recurrent infections, including chronic otitis media, upper respiratory infections, severe or progressive pneumonia, and cutaneous inflammatory lesions. While the majority of documented infections were bacterial in origin, these individuals also were susceptible to viral infections, reflecting in part, impairment of cell-mediated immunity in this disease. One patient died of overwhelming adenoviral pneumonia.[333] A diagnosis of mannosidosis as suggested by typical clinical features can be confirmed by the demonstration of deficient acidic α-mannosidase activity in plasma, peripheral blood leukocytes, or cultured skin fibroblasts.

Periodontitis Syndromes

Experimental and clinical evidence has documented the important protective role of phagocytic cells, and in particular, neutrophils in tissues of the oral cavity.[334] The infiltration of neutrophils into gingival tissues early in the development of gingivitis is thought to provide a first-line defense against invasion by pathogenic oral microflora.[335] Individuals with developmental, genetic, or acquired disorders characterized by quantitative deficiencies of peripheral blood phagocytes or functional abnormalities of neutrophils commonly present with oral complications.[26,117,132,231,314,334–342] Primary or secondary agranulocytosis, and cyclic neutropenia syndromes are typified by severe ulceration, necrosis, or chronic inflammation of gingival or periodontal tissues.[337] Patients with severe leukocytopathies such as chronic granulomatous disease,[341] Chediak-Higashi syndrome,[339] and leukocyte adhesion deficiency[132] present with systemic as well as oral infections while those demonstrating less profound functional deficits such as in localized juvenile periodontitis, postlocalized juvenile periodontitis or generalized juvenile periodontitis present exclusively with periodontal manifestations.

Defective chemotactic responsiveness of neutrophils is thought to represent a major pathogenic mechanism in individuals with periodontitis syndromes.[248,334,340,343–349] Of 183 patients with localized juvenile periodontitis studied by multiple investigators[329,334,338,345,347,349–351] 132 (71 percent) have been reported to exhibit defective chemotaxis. Most patients exhibit intrinsic cellular defects, but cell-directed serum inhibitors, chemotactic factor inactivators, or abnormalities of chemotaxigenesis have been reported in a small proportion of patients tested. The pathogenic mechanisms accounting for impaired chemotaxis have not been defined. The epidemiologic or clinical associations of certain periodontopathic bacterial organisms with some periodontitis syndromes have suggested the possibility that cellular constituents or extracellular factors elaborated by these microorganisms may secondarily alter functions of leukocytes.[336,352–356] The pathogenic roles of gram-negative oral bacteria including *Actinobacillus actinomycetemcomitans*, species of *Bacteroides*, and species of *Capnocytophaga* have been increasingly appreciated.[352,356] Among the potentially pathogenic products of *A. actinomycetemcomitans*, a leukocytotoxin has been identified in vitro which may contribute to diminished chemotactic function.[357–360] Serum from selected juvenile periodontitis patients contains IgG antibodies which neutralize leukotoxic activity of *A. actinomycetemcomi-*

tans, and serum and gingival crevicular fluids from such patients contain high titers of antibodies to *A. actinomycetemcomitans* antigens.[360] While the molecular basis of leukotoxin production or toxicity remains undefined, the development of techniques to isolate leukotoxin from *A. actinomycetemcomitans*[361] and the availability of antileukotoxin monoclonal antibodies should facilitate and expand studies concerning its pathogenic role in localized juvenile periodontitis.[362] Other poorly defined inhibitors of chemotaxis have been found in culture filtrates or sonicates of *Bacteroides gingivalis*, *Fusobacterium nucleatum*, and species of *Capnocytophaga*.[336,354]

Despite intensive study, the prevalence, natural history, and etiology of juvenile periodontitis remain undefined. The familial aggregation in juvenile periodontitis has prompted a number of researchers to propose a possible genetic basis for this disease.[342,363] Defects of chemotaxis associated with juvenile periodontitis also appear to have a familial pattern of distribution, and in some cases both functional defects of leukocytes and clinical features of periodontitis are identified in multiple family members.[342] It has not, however, been determined if the familial occurrence of juvenile periodontitis results from a single major gene, multifactorial etiology, or environmental effects. Conflicting reports suggest an autosomal recessive or X-linked dominant mode of inheritance.[363–365] The mode of genetic transmission of specific periodontitis syndromes will await the identification of molecular markers for disease.

chwachman-Diamond Syndrome

inical features of a syndrome first described by Schwachman d Diamond include exocrine pancreatic insufficiency, bone urrow hypoplasia with associated neutropenia, metaphyseal ondrodysplasia, growth retardation, and recurrent soft tis- infections.[366–369] In a series of 21 patients,[369] otitis media, nchial pneumonia, osteomyelitis, dermatitis, and septi- ia occurred in 17 (85 percent) of whom 3 (15 percent) l. Neutropenia was intermittent in most patients in this other series.[370] Bone marrow aspirations in this disorder e demonstrated absent myeloid precursors or maturation st with variable degrees of hypoplasia.[368–370] Normal bone row aspirates in neutropenic patients have also been de- ed, suggesting that marrow hypoplasia is patchy in distri- on.[367] Diminished chemotaxis of neutrophils without other tional abnormalities was found in 12 of 14 patients with syndrome.[366] Nine of these patients were neutropenic, and demonstrated low levels of serum IgA or IgM without immunologic abnormalities. Intermediate abnormalities utrophil chemotaxis were recognized in parents of some ese individuals suggesting that they were heterozygotes hat this abnormality is inherited as an autosomal reces- lisorder. A pathogenic basis for hematologic as well as features of this multisystem disease has not been deter- l, and the relative contributions of impaired cellular mo- as opposed to neutropenia to infectious susceptibility in d patients is uncertain.

ders Associated with Abnormal Generation of otactic Factors

mal chemotaxigenesis occurs in a number of genetic or ed disorders, most importantly those associated with ab- lities of serum complement proteins.[213,371,372] (See Chap. 111 for detailed discussion of abnormalities of complement.) Diminished generation of complement-derived chemotactic factors (primarily C5a and $C5a_{desArg}$), has been demonstrated in association with a heritable deficiency of complement components C3 and C5,[373–376] and in a patient with Klinefelter syndrome who displayed a congenital absence of a C3 regulatory protein normally present in plasma.[373] Impaired generation of chemotactic activity in serum has also been described in a 19-year-old female patient with C5 deficiency and systemic lupus erythematosus.[377] Abnormal generation of complement-dependent chemotactic activity was found in sera of 10 of 23 patients with systemic lupus erythematosus, and this was thought to contribute to infectious susceptibility in some cases.[378] Two children with a clinical syndrome of dermatitis, diarrhea, wasting, dystrophy, and recurrent pyogenic infections (Leiner disease) were reported to have C5 dysfunction and reduced chemotactic activity.[379]

Individuals with these abnormalities of serum complement are susceptible to recurrent localized soft tissue infections as well as systemic episodes (septicemia, meningitis, septic arthritis) secondary to staphylococci, gram-negative enteric bacteria, *C. albicans*, and encapsulated pathogens, including *Streptococcus pneumonia*, and *Haemophilus influenzae* type b. Since they demonstrate severely diminished serum opsonic and/or serum bactericidal activities, the relative contributions of impaired chemotaxigenesis to their infectious susceptibility is uncertain. The preferential importance of complement components C3 and C5 for chemotaxigenesis is emphasized by the findings of normal generation of chemotactic activity in serum from patients with inherited deficiency of C6 or C8.[371] Early classic pathway complement components appear to be required for optimal chemotactic factor generation but only at limiting concentrations of serum. Moreover, individuals with heritable C2 or C4 deficiency generally do not demonstrate increased susceptibility to infectious diseases.[371]

Similar complex abnormalities of serum complement-mediated functions (including chemotaxigenesis) have been reported in human neonates,[296,300,380] and children with nephrotic syndrome who lose serum factor B in the urine.[381] Abnormal generation of chemotactic activity on incubation of serum with endotoxin has been reported in several patients with hypogammaglobulinemia,[371] although the basis for this defect is not elucidated. Abnormalities in the formation of the Hageman factor-dependent chemotactic agents kallikrein and plasminogen activator have also been demonstrated in plasma of patients with genetic deficiency of Hageman factor[213] or prekallikrein.[382] Normal serum contains inhibitors that are capable of destroying the biologic activity of a variety of chemotactic factors by enzymatic cleavage.[7] High levels of these inhibitors have been described in several groups of patients among whom the incidence of infections is increased. These include patients with renal diseases during chronic hemodialysis,[383] Hodgkin's disease,[384] lepromatous leprosy,[385] sarcoidosis,[386] and cirrhosis.[387]

Defects in Motility Associated with Immunologic Disorders

Primary immunodeficiency syndromes are described in Chaps. 109 and 110. In some of these disorders, abnormalities of cellular motility have been described. The contributions of these abnormalities to infectious susceptibility in each is uncertain. Possibly the first report of a clinical defect of chemotaxis[6] was

a description of two female patients with fair skin, reddish hair, severe eczema, dystrophic fingernails, sinopulmonary infections, and recurrent staphylococcal abscesses (termed *Job syndrome*). Inflammatory lesions of soft tissues in these patients, despite their considerable size, demonstrated minimal erythema or tenderness. In 1972, two male patients with essentially the same syndrome were described.[9] The patients exhibited peculiar coarse facies, eczematoid rashes, cold abscesses, and recurrent sinopulmonary infections due to *S. aureus* or *H. influenzae*. Both demonstrated hyperimmunoglobulin E and a variety of additional subtle immunologic abnormalities. A summary of the clinical courses of 20 patients with hyperimmunoglobulin E revealed 13 males and 8 blacks, thus eliminating the concept that Job syndrome affects only red-haired females.[388] All had eczematoid dermatitis, and in seven instances a familial occurrence was noted. Serum IgE levels in unaffected relatives were normal. The patients consistently demonstrated poor delayed hypersensitivity responses as well as poor anamnestic responses to tetanus and diphtheria antigens. Almost all demonstrated diminished lymphocyte proliferation in vitro to specific antigens such as *C. albicans* or tetanus toxoid, but proliferative responses to lectins were generally normal. Other reports documented deficient suppressor T lymphocytes and increased IgE synthesis in culture.[389,390] Collectively, these reports suggest a defect of immune regulation as the primary pathogenic basis of this syndrome.[391] Heterogeneity with respect to the chemotactic functions of neutrophils or monocytes from patients with Job syndrome has lead to the consideration that these abnormalities do not reflect a primary dysfunction of neutrophils or monocytes.[11,392] Rather, these abnormalities may be related to high tissue levels of histamine. Histamine significantly inhibits the chemotactic response of normal neutrophils in vitro. Cytophilic IgE directed against invading bacteria could mediate a local release of histamine, thereby diminishing chemotaxis of circulating neutrophils. Patients with Job syndrome have been found to have high levels of serum IgE directed against antigens of *S. aureus* and *C. albicans*.[393,394]

One large patient group that must be carefully differentiated from individuals with Job syndrome includes those individuals with atopic eczema who are frequently colonized by *S. aureus* and later acquire secondary staphylococcal infections. These patients may demonstrate chemotactic defects,[395] but generally they do not demonstrate recurrent sinopulmonary infections and characteristic cold abscesses. Still other patients with a prominent allergic history develop recurrent infections that coincide with exacerbations of atopic symptoms.[396]

Defects in chemotaxis have also been described in selected patients with chronic mucocutaneous candidiasis, and these may accompany or occur in the absence of associated lymphocyte dysfunction.[10,214,397,398] Diminished chemotaxis of neutrophils associated with marked elevation of IgE was reported in one mother-daughter pair,[399] and abnormal mononuclear leukocyte chemotaxis associated with abnormal production of lymphocyte-derived chemotactic factor was reported in another patient.[214] A plasma inhibitor of neutrophil motility was detected in one patient with chronic mucocutaneous candidiasis. Partial characterization of this inhibitor revealed that it had several properties in common with IgG.[398]

A cellular defect of chemotaxis, phagocytosis, and intracellular bactericidal activity was reported in a 3-year-old male with agammaglobulinemia, recurrent cutaneous abscesses, and episodes of pneumonia. Similar defects were found in an adult with hypogammaglobulinemia and in another child with gamma globulin deficiency associated with recurrent sinopulmonary infections.[213] Diminished chemotaxis occurred in 9 of 10 patients with serum IgA deficiency and 6 of 10 patients with hypogammaglobulinemia.[400,401] Diminished chemotaxis has also been observed in selected patients with severe combined immune deficiency disease.[402] The pathogenic basis and consequences of abnormal cellular motility described in these reports are uncertain.

In addition to complex immunologic abnormalities identified in patients with the Wiskott-Aldrich syndrome, diminished chemotaxis of monocytes has been reported in association with abnormal production of a lymphocyte-derived chemotactic factor.[403] These findings suggest that lymphocytes in Wiskott-Aldrich syndrome may release soluble factors that diminish the responsiveness of monocytes to chemotactic stimuli. The pathogenic significance of these limited findings is uncertain.

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