An Inherited Defect of Neutrophil Motility and Microfilamentous Cytoskeleton Associated With Abnormalities in 47-Kd and 89-Kd Proteins

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A 2-month-old male Tongan infant presented with fever, severe skin and mucosal infections, hepatosplenomegaly, thrombocytopenia, and normal neutrophil counts. While polymorphonuclear neutrophil (PMN) morphology was normal, several neutrophil motile functions were found to be altered in the patient. Furthermore, two siblings had died in infancy with a similar clinical picture, raising the possibility of an inherited neutrophil defect. Random migration and chemotaxis, assessed by the agarose method, were profoundly impaired. Actin polymerization, as measured by flow cytometry of N-[7-nitrobenz-2-oxa-1,3-diazol-4-y]phallacidin (NBD-phallacidin)-stained PMNs, showed lower basal F-actin and a 1.75-fold increase in response to 10⁻⁷ mol/L formyl-methionyl-leucyl-phenylalanine (FMLP) compared with a 4.51-fold increase in control. Microscopic examination of NBD-phallacidin-stained PMN spread on glass showed decreased area of spreading and F-actin–rich filamentous projections distinct from control. The early phase of FMLP-induced right angle light scattering was absent, similar to the effect caused by cytochalasin-B (CB), an inhibitor of actin polymerization. Accordingly, FMLP induced secretion of elastase without the addition of CB. *Staphylococcus aureus* killing was 50% of control whereas superoxide production in response to FMLP and surface expression of CD11b were greater than twice normal. Partial defects in actin polymerization and scatter were seen in the parents and release of elastase, in the absence of CB, was also increased in both parents. Sodium dodecyl sulfate-polyacrylamide electrophoresis of whole cell proteins from the patient showed a marked decrease in an 89-Kd protein (8% of control) and a marked increase in a 47-Kd protein (4.2-fold). Both mother and father had decreased 89-Kd (77% and 42% of control) and increased 47-Kd proteins (2- and 3.4-fold), although neither had recurrent infections or chemotactic defects. These studies describe a new inherited actin dysfunction syndrome associated with severe propensity to fungal infection and draw attention to the proteins of apparent molecular weights of 89 Kd and 47 Kd, which may be of great importance in the regulation of actin polymerization in human PMNs.

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CIRCULATING polymorphonuclear neutrophils (PMNs) sense bacteria in tissue, adhere to endothelium, and then move toward, ingest, and kill the bacteria. This mobilization of PMNs to sites of infection requires intact surface receptors for bacteria-derived attractants, surface adhesion molecules, and signal transduction events that convert the surface stimulus into biochemical responses that produce the force for motility and the microbicidal agents for killing. Severe defects in neutrophil adherence, motility, or intracellular killing result in recurrent, often life-threatening bacterial infections in humans.¹,³ Stimulus-induced reorganization of the microfilamentous cytoskeleton and its basic structural element, filamentous actin (F-actin), is critical for most motile behaviors of PMNs including shape change, locomotion, chemotaxis, phagocytosis, secretion, and may influence other functions such as superoxide (O²⁻) production.⁴ Evidence that links the integrity and dynamics of microfilamentous cytoskeleton to neutrophil motility derives primarily from studies of the effect of cytochalasins on neutrophil motility⁷ and case reports of patients with defective microfilamentous cytoskeletal dynamics in PMNs.⁵⁶ Cytochalasins are alkaloids that can block the polymerization of monomeric actin (G-actin) to polymeric F-actin, sever actin filaments, and inhibit PMN locomotion, chemotaxis, phagocytosis,¹⁰,¹¹ and dramatically alter cell shape. In addition, disruption of the F-actin–rich, subcortical cytoskeleton in cytochalasin-treated PMNs or in genetically defective PMNs enhances secretion and superoxide release.¹² Evidence suggests that this enhancement may be due to facilitation of granule-plasma membrane fusion³ or to direct effects of the cytochalasins on the interactions of receptors and NADPH oxidase components with the microfilamentous cytoskeleton.³⁰,¹²

Elucidation of the biochemical mechanisms for regulation of the microfilamentous cytoskeletal organization and actin polymerization and the consequences of such regulation for the function of nonmuscle cells such as neutrophils is the focus of intense current research.¹³ In recent years, investigators have identified and characterized a variety of proteins that are present in small quantities in phagocytes and regulate actin polymerization in vitro. These include profilin, gelsolin, actin-binding protein, and related proteins.¹⁴,¹⁵ Growing evidence suggests that these proteins also play a role in regulating cytoskeletal organization in PMNs.¹⁶,¹⁷ However, much is yet to be learned about the role of these proteins in regulating actin polymerization in PMNs. The description of patients with motility-deficient PMNs and the biochemical elucidation of such defects are critical to continued growth in our understanding of the role of these proteins and of the cytoskeleton in normal and abnormal PMN function.

This report describes a patient with severe recurrent infections, defects in PMN motile behaviors, and defective actin polymerization and microfilamentous cytoskeletal organization in basal and activated PMNs. The abnormalities in PMN function and microfilamentous cytoskeletal

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organization are associated with decreased amount of an 89-Kd protein and increased amount of a 47-Kd protein (apparent molecular weights, MW) in the PMNs. Clinical, functional, and biochemical studies in the patient and the parents show that this is a novel inherited disorder of neutrophil motility and suggest that either or both the 89-Kd and the 47-Kd proteins play an important role in regulating microfilamentous cytoskeletal organization and motile functions of human PMNs.

MATERIALS AND METHODS

Cell preparation. Heparinized venous blood was obtained from the patient, family, healthy adults, and age-matched donors in accordance with the Helsinki declaration and with approval of the Human Experimentation Committee of Childrens Hospital Los Angeles. PMN were prepared by dextran sedimentation and Ficoll-Hypaque centrifugation (Winthrop Pharmaceuticals, New York, NY; Sigma Chemicals, St Louis, MO). Erythrocytes were removed by hypotonic lysis.

Reagents. Stock solutions of formyl-methionyl-leucyl-phenylalanine (FMLP; Sigma Chemical, St Louis, MO) were prepared at 10^4 mol/L in 1% dimethyl sulfoxide (DMSO) and kept frozen. Medium 199 2X containing Earle's modified salts, penicillin (100 U/mL), and streptomycin (100 mg/mL) (GIBCO, Grand Island, NY) was used to prepare the agarose gel. Medium 199 1X, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, was used for cell suspension. Electrophoresis-grade agarose, 15 mg/mL (J.T. Baker Chemical Co, Phillipsburg, NJ) was dissolved in sterile distilled water by heating. N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phallacidin (NBD-phallacidin) and MeO-Suc-Ala-Pro-Val-methylcoumarin amide were obtained from Molecular Probes Inc (Junction City, OR). Reagents for electrophoresis were from BioRad (Richmond, CA). All other reagents were from Sigma or as indicated.

Neutrophil functions. Chemotaxis was assayed using a modification of the under agarose technique described by Nelson et al. The leading front (LF) and random migration (RM) were measured from the cell migration pattern using a video imaging system (502A; American Type Culture Collection [ATCC], Rockville, MD). Monocyte contamination was excluded by double staining with Leu-M3 (Becton-Dickinson). The rate of spreading was determined by allowing PMN at 1 x 10^6 per mL in Krebs Ringers phosphate glucose (KRP) to settle at 37°C on glass coverslips. The slides were fixed and stained with NBD-phallacidin at 5 and 15 minutes and viewed by phase/fluorescence microscopy.

The cell area was determined by video image analysis. Surface CD11b was measured by flow cytometry (FACScan; Becton-Dickinson, Mountain View, CA) using MO-1 antibody (Coulter, Hialeah, FL). Monocyte contamination was excluded by double staining with Leu-M3 (Becton-Dickinson).

F-actin quantitation. PMNs were treated with DMSO or stimulus for the indicated times at 37°C or 25°C, fixed, and stained with a cocktail containing lysophosphatidylcholine (100 μg/mL) and NBD-phallacidin (3.3 x 10^4 mol/L) as previously described. Fluorescence was quantitated by flow cytometry. To compare the F-actin content measured on different days in family members, all values were normalized to the maximum F-actin content from the control cells used on the same day.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and gel scanning. PMNs were treated on ice for 10 minutes with 5 mmol/L diisopropyl fluorophosphate (DFP) and whole cell proteins were solubilized by boiling in 2% SDS, 20% glycerol, 10% β-mercaptoethanol, 6 mmol/L DFP, 0.125 mol/L Tris-HCl pH 6.8. Samples were kept frozen at ~70°C until use. After thawing, the samples were sonicated and the protein concentration was determined using a modification of the method of Lowry after precipitation with 10 vol of 10% perchloric acid/1% phosphotungstic acid. Bovine serum albumin was used as the standard. Equal loads of whole cell proteins were analyzed on 5% to 15% polyacrylamide gradients as described by Laemmli. To quantitate the relative amounts of protein on the one-dimensional Coomassie blue-stained gels, the intensities of the 47-Kd, 89-Kd, and actin bands were measured in optical density (OD) units by laser scanning densitometry (Ultrascan; LKB Biotechnology, Uppsala, Sweden) as previously described.

Data analysis. Comparisons of group means were tested by analysis of variance or the Student's t-test. The results presented are representative of at least three experiments. Except where otherwise noted, the specific data points represent the mean and standard deviation of a triplicate determination on a single donor.

RESULTS

Clinical data. A 2-month-old male Tongan infant was referred to Childrens Hospital Los Angeles with recurrent fevers of several weeks duration. Physical examination showed an ulcer of the hard palate, hepatosplenomegaly, bruising, and petechiae. During the ensuing 3-month hospitalization, he developed recurrent pulmonary infiltrates, progression of the hard palate lesion, and a subsequent lingual ulcer. Culture of the lingual ulcer grew Candida tropicalis. Repeated blood cultures were negative. Episodes of pulmonary infiltrates resolved on broad-spectrum antibiotics. The oral ulcers resolved slowly on amphotericin-B.

The pregnancy and delivery were uncomplicated with no history of maternal drug ingestion. There was no history of delayed separation of the umbilical cord. The child has seven siblings, two of whom died at 3 and 4 months of age as the result of complications of recurrent oral, perirectal, and pulmonary infections. The deceased infants also had bruising similar to that of the patient. The surviving siblings have no significant medical problems nor is there a history of severe recurrent infection on either side of the family. There are no family records that permit determination of the exact blood relation between the mother and father. However, the parents were not brother and sister and it is...
likely that the maternal and paternal great-grandparents were closer than fourth cousins.

The patient’s hemogram showed a white blood cell (WBC) count of 16,000/mm$^3$ with a normal differential and a platelet count of 64,000/mm$^3$. On Wright’s stain peripheral blood smear, the nuclear morphology of the PMNs was normal and the cytoplasm contained neutral granules but no giant granules. The hemoglobin was 9 g/dL and there was mild elevation of the liver enzymes. Serum Igs, Ig subclasses, response to immunization with tetanus, T-cell numbers, and phytohemagglutinin (PHA) stimulation were normal. Bone marrow aspiration showed normal granulocyte morphology and maturation as well as normal megakaryocyte number and morphology. Of note, the patient had a normal response to transfused platelets, suggesting that the thrombocytopenia was not due to increased platelet destruction. Examination of transmission electron micrographs of neutrophils fixed in suspension was normal and demonstrated normal specific granule morphology. Bone marrow chromosomes as well as peripheral lymphocyte chromosomes were normal. Subsequent evaluation of neutrophil function (detailed below) showed a profound defect in chemotaxis, normal or increased superoxide production, and increased expression of CD11b.

During the hospitalization, the palatal ulcer worsened and cultures of a lingual ulcer grew *Candida* and *Aspergillus niger*. The child was treated with antibiotics and amphotericin-B with slow resolution of the infectious problems. At 7 months of age, the child underwent allogeneic bone marrow transplantation from an HLA-identical sibling with resultant correction of the thrombocytopenia and neutrophil chemotactic defect.

**Neutrophil motility and related functions.** Direct observation with phase microscopy of the patient’s PMNs in a gradient of FMLP (Fig 1B) showed occasional attempts at pseudopod formation, and frequent development of thin, filamentous projections at the cell surface while control cells formed broad pseudopods. However, the patient’s PMNs did not locomote or orient in the FMLP gradient, when compared with control PMNs. In quantitative motility assays, random migration and chemotaxis toward FMLP were markedly impaired when compared with normal adults or to age-matched controls (Fig 2). Results were similar with leukotriene B$_4$ as the chemotactant (data not shown). Control PMNs exposed to the patient’s serum exhibited normal chemotaxis while exposure of patient’s PMN to normal serum did not improve the defective chemotaxis or random migration, indicating a defect intrinsic to the patient’s PMNs. Direct observation of vital maternal and paternal PMNs showed no qualitative morphologic abnormality and quantitative studies of chemotaxis and random motility were normal.

The abnormalities in PMN motility were associated with an altered FMLP-induced right angle light scatter. An early decrease in right angle light scatter is observed after FMLP stimulation of normal PMNs and correlates with shape change and with actin polymerization.$^{27}$ When compared with controls, the expected early decrease, measured at 37°C, was significantly diminished in the patient’s PMNs (Fig 3). The diminished right angle light scatter response could relate to either an inability of the cells to change shape or to a defect in FMLP-induced actin polymerization. The early response in right angle light scatter after stimula-

![Fig 1. Morphology of PMNs during movement in a gradient of FMLP. Normal cells viewed by phase microscopy at 400× magnification (A) orient and move in the gradient whereas the patient’s cells (B) remain round and produce hairlike pseudopods.](image)

![Fig 2. Motility of control, patient, and parent PMNs. Random migration (■) and chemotaxis as measured by leading front (○) in response to $10^{-7}$ mol/L FMLP in 30 adult controls (A), two age-matched infant controls (C), patient (PT; n = 3), father (F; n = 3), and mother (M; n = 3).](image)
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Figure 3. Shape change in control and family PMNs. Early changes in right angle scatter after stimulation with 10⁻⁷ mol/L FMLP in control (C), father (F), mother (M), and patient (PT) PMNs. The stimulus was added at 0 seconds. Each line is the mean of three replicates on a representative day.

Figure 4. Spreading of control and patient PMNs on glass. PMNs were fixed after spreading on glass for 15 minutes at 37°C. Control PMNs are seen on the left and patient's PMNs are seen on the right. Patient's PMNs (C) fail to demonstrate punctate regions of F-actin as seen in normal control (A) and have thin F-actin-rich filamentous projections that are not present in normal PMNs. These filaments are closely adherent to the glass and appear as black lines by reflectance-interference microscopy (D).

Microfilamentous cytoskeleton and actin polymerization. Microscopic observation of patient and control PMNs that had spread on glass coverslips for 15 minutes at 37°C showed striking morphologic changes (Fig 4, B and D). Phase contrast microscopy and reflectance interference microscopy demonstrated that few cells spread significantly and that 20% of the patient's PMNs expressed fine, hairlike projections from their surface when the cells attempted to spread on a surface (Fig 4D). These thin filamentous projections were barely visible by phase microscopy (Fig 1B); however, by reflectance interference microscopy, the projections appeared as thin dark lines, suggesting close adhesion of the projections to the glass substratum (Fig 4D). These filamentous thin projections were not seen on electron micrographs of cells fixed in suspension (data not shown). Fluorescence microscopy of cells stained with NBD-phallacidin, a specific probe for F-actin, showed that the thin filamentous projections were F-actin-rich as evidenced by the intense fluorescence (Fig 4B). The patient's PMNs stained with NBD-phallacidin also lack the punctate sites of localized F-actin concentration that were observed in normal PMNs adherent to glass in the absence of protein (Fig 4A).

The basal F-actin content and FMLP-induced actin polymerization response of PMNs from the patient were determined by flow cytometric analysis of NBD-phallacidin-stained PMNs. Maximum FMLP-induced actin polymerization occurs after 15 seconds of incubation and is defined as the maximum F-actin content. The results with the patient's PMN were expressed as percent of the maximum F-actin content obtained with control cells. As shown in Fig 5A, both basal F-actin content and increase in F-actin content after FMLP stimulation were markedly decreased in the patient's PMNs as compared with control PMNs (P < .001; Fig 5A). The F-actin content and polymerization of two age-matched controls with decreased chemotaxis did not differ from adult controls. The maximum extent of FMLP-induced actin polymerization in PMNs of both
stimulation with present both at 37°C (Fig 5A) and 25°C (Fig 5B). More detailed kinetics of F-actin change after stimulation with 10^{-7} mol/L FMLP at 25°C in control (△), father (●), and mother (▲). All points are normalized to the maximal F-actin content of the intermediate defects in the mother's and father's PMNs induced the release of large amounts of vitamin B_{12} binding protein and the production of significant amount of O_{2}^- without prior exposure to cytochalasins. Furthermore, surface expression of CD11b, a membrane-associated secondary granule component, was upregulated in the patient's basal PMNs, ie, in the absence of stimulus or cytochalasin B (CB). These findings suggest that the secretion of secondary granules is facilitated in the patient's cells without addition of CB. Surprisingly, stimulation of the patient’s PMNs with FMLP induced significant release of the primary granule constituent elastase, in the absence of added CB (Table 1). The data on secretion and O_{2}^- production suggest that the cytoskeletal defect in the patient’s PMNs mimics the effects observed in cytochalasin-treated cells. Interestingly, PMNs from both parents also exhibited FMLP-induced elastase release without exposure to cytochalasins (Table 1).

Electrophoretic studies of PMN proteins. Total cellular protein from neutrophils of the patient and the parents was analyzed by one-dimensional SDS-PAGE (1D-SDS-PAGE). Results are shown in Fig 6 and Table 2. SDS-PAGE of the patient’s PMNs showed a marked increase in a 47-Kd protein while the amount of an 89-Kd protein was decreased. The decrease in 89-Kd protein was most obvious in gels heavily loaded with total cellular protein (Fig 6A).

Table 1. Secretion-Dependent Functions

<table>
<thead>
<tr>
<th>Function</th>
<th>Control</th>
<th>Patient</th>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b* (fold control)</td>
<td>1.0</td>
<td>2.13</td>
<td>1.77</td>
<td>1.56</td>
</tr>
<tr>
<td>Superoxide (nm/min/10^6 PMN)</td>
<td>132 ± 7.5</td>
<td>416 ± 6.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FMLP 10^{-7} mol/L</td>
<td>260 ± 4.8</td>
<td>426 ± 3.8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Vitamin B_{12} binding protein† (% release)</td>
<td>15.8 ± 1%</td>
<td>36 ± 2.3%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FMLP 10^{-7} mol/L</td>
<td>23.0 ± 1.6%</td>
<td>38 ± 1.2%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PMA 50 ng/mL</td>
<td>46.7 ± 3.2%</td>
<td>52 ± 2.0%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Elastase release† (fold baseline)</td>
<td>0.99 ± 0.5</td>
<td>5.4 ± 1.0</td>
<td>5.2 ± 1.7</td>
<td>1.7 ± 0.56</td>
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</table>

*Unstimulated mean channel number compared with control.
†Differences between patient and control are significant at P < .01. The effect of CB was not significant in the patient.
‡The fold increase of the rate of change in fluorescence 1 minute after stimulation compared with baseline. The P values indicate difference from baseline.
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SDS-PAGE of the parent’s PMNs also showed decreased amounts of the 89-Kd protein and increased amounts of the 47-Kd protein. As shown in Table 2, quantitative scans of replicate gels demonstrated that the magnitude of the decrease in 89-Kd protein was less marked in the father and the mother than in the patient. Specifically, the amount of 89-Kd protein in the mother, father, and the patient was 77%, 42%, and 8% of control, respectively. The increase in 47-Kd protein was statistically significant in PMNs from the mother, father, and patient (2.0-fold, 3.4-fold, and 4.2-fold, respectively). Similar qualitative changes in 89-Kd and 47-Kd proteins in PMNs from the patient and the parents and the decrease in 89-Kd bands (arrowheads).

Table 2. Relative Quantities of 47-Kd and 89-Kd Proteins in Neutrophils

<table>
<thead>
<tr>
<th>OD 47 Kd*</th>
<th>47 Kd: Actin Ratio</th>
<th>OD 89 Kd*</th>
<th>89 Kd: Actin Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.035 ± 0.015</td>
<td>0.21 ± 0.11</td>
<td>0.184 ± 0.015</td>
</tr>
<tr>
<td>Patient</td>
<td>0.146 ± 0.030</td>
<td>1.00 ± 0.15</td>
<td>0.015 ± 0.006</td>
</tr>
<tr>
<td>Mother</td>
<td>0.073 ± 0.020</td>
<td>0.75 ± 0.12</td>
<td>0.143 ± 0.038</td>
</tr>
<tr>
<td>Father</td>
<td>0.120 ± 0.023</td>
<td>0.80 ± 0.10</td>
<td>0.078 ± 0.009</td>
</tr>
</tbody>
</table>

The amount of actin in equally loaded gels from parents and patient was the same as control.
*OD determined by gel scan (n = 3) on gels of equal total protein load (micrograms).
†Ratio of OD 47 Kd or 89 Kd to OD actin (ie, independent of protein load; n = 4).

suggest that alterations in the amounts of 47-Kd and 89-Kd proteins may be inherited as an autosomal recessive trait.

DISCUSSION

This communication reports the clinical and biochemical studies of a Tongan infant who presented with severe, recurrent infections. Functional studies of the patient’s neutrophils showed abnormalities in locomotion, phagocytosis, shape change, and secretion that were associated with a defect in microfilamentous cytoskeletal organization and actin polymerization. Clinically, the patient had normal total and differential leukocyte counts, marked thrombocytopenia with normal survival of transfused platelets, and a propensity to infection. Morphologically, by light microscopy, the patient’s PMNs failed to form pseudopods, but developed long, filamentous, and F-actin–rich projections during attempts at spreading. Because several motility-related PMN functions were significantly depressed, the basal F-actin content and FMLP-induced actin polymerization responses of the patient’s cells were analyzed by NBD-phallacidin fluorescence. Both basal and stimulated F-actin content were markedly decreased, suggesting that the defects in motility are related to abnormalities in microfilamentous cytoskeletal structure and dynamics. Quantitative scanning of 1D-SDS-polyacrylamide gels showed normal actin content in the patient’s PMNs; however, proteins of apparent MW 47 and 89 Kd were profoundly abnormal. The association of a defect in motility and actin polymerization with abnormalities in proteins of 47 and 89 Kd apparent MW raise the possibility that these proteins play a role in regulating the organization of the microfilamentous cytoskeleton in PMNs.

Secretion and O2 release, functions that are not usually thought to require an intact microfilamentous cytoskeleton, were also found to be abnormal in the patient’s PMNs. On stimulation with FMLP, normal PMNs do not release primary granule constituents and exhibit limited O2 production or specific granule release. However, after pretreatment of normal PMNs with cytochalasins, which block actin polymerization, FMLP induces the release of primary granule components like elastase and causes increased...
release of specific granule constituents such as vitamin B<sub>12</sub> binding protein, increased surface membrane expression of CD11b that is stored in the specific granules, and enhancement of the respiratory burst, components of which are located in specific granules. Possible explanation for this enhancement includes upregulation of surface receptors through membrane addition and cytochalasin-induced dissolution of the subcortical microfilaments. PMNs from the patient were functionally similar to cytochalasin-treated PMNs in that they exhibited increased surface CD11b, enhanced O<sub>2</sub><sup>-</sup> production to FMLP, increased vitamin B<sub>12</sub> binding protein release that was not further enhanced by cytochalasins, and release of elastase in the absence of cytochalasins. Furthermore, the patient’s PMNs share morphologic features with cytochalasin-treated PMNs. Specifically, the fine hairlike projections from the patient’s adherent PMNs recall the projections produced following cytochalasin-induced PMN “arborization”. These abnormalities in secretion and O<sub>2</sub><sup>-</sup> production, though not as directly related to cytoskeletal function as locomotion and phagocytosis, support the idea that the patient’s PMNs have an intrinsic defect that causes microfilamentous cytoskeletal dysfunctions similar to those reported in cytochalasin-treated PMNs.

Partial expression of the functional and biochemical phenotype was observed in the patient’s PMNs. While PMN chemotaxis, which was profoundly depressed in the patient, was normal in both parents, FMLP-induced actin polymerization response and right angle light scatter were significantly decreased in both parents. Furthermore, PMNs from both parents released significant amount of elastase in the absence of cytochalasin. In general, the magnitude of the abnormalities was greater in the mother’s than in the father’s PMNs. Interestingly, the functional phenotype of parents’ cells does not directly correlate with the extent of the increase in 47-Kd or decrease in 89-Kd proteins in parental cells. Both parents differ from control in the relative amounts of 47-Kd and 89-Kd proteins. The father has significantly less 89-Kd protein and more 47-Kd than the mother, and yet the abnormalities in PMN functions are less severe in the father’s PMNs. However, the functional and biochemical data on PMNs, the history of affected siblings, and the parents’ consanguinity clearly indicate that the PMN defect is inherited and is likely autosomal recessive.

Severe congenital deficiencies of PMN locomotion and chemotaxis are usually associated with a marked propensity to development of infections and include several rare disorders such as Chediak-Higashi syndrome, specific granule deficiency, and leukocyte adhesion deficiency (LAD). These were excluded in this patient based on the normal morphology of Wright-stained PMNs, the presence of specific granules on electron micrographs, and the increased expression of CD11b. Another motility disorder, named neutrophil actin dysfunction (NAD), was described earlier by Boxer et al and subsequently was partially characterized through biochemical studies on the family. Some features are shared by both patients, namely profound motility abnormalities, abnormal actin polymerization, and increase in spontaneous secretion, and the parents of both patients have partial impairment of actin assembly. However, several distinct clinical, functional, and biochemical differences in the patients and their families also exist. Clinically, the NAD patient had a marked neutrophil leukocytosis (> 100,000/mm<sup>3</sup>), normal platelet counts, and predominantly bacterial infections. Morphologically, at the light and electron microscopic level, PMNs from the NAD patient formed pseudopods that were “fork-like.” Biochemically, crude extracts of the NAD patient’s PMNs failed to polymerize actin normally, but SDS-PAGE of whole cells was normal. Analysis by 1D-SDS-PAGE of Triton-insoluble cytoskeletons showed no abnormality in the father’s PMNs but showed increased amounts of a 54-Kd protein in PMNs from the mother and one of the siblings. The mother and the older sister were found to be partially deficient for both CD11b and CD18, the two subunits of the glycoprotein receptor CR3, suggesting that they were heterozygotes for LAD in association with NAD. However, the father had nearly normal expression of both subunits. In contrast to the NAD patient, the functional and biochemical analysis of the patient’s and parent’s PMNs described here suggest that this patient is homozygous for an autosomal defect that is associated with not only an increased amount of a 47-Kd protein but also a decrease in an 89-Kd protein. These distinctions indicate that this patient may represent a unique disorder of PMN motility and function which, like NAD, manifests abnormalities in the microfilamentous cytoskeleton and ligand-induced actin polymerization responses. Because the NAD patient and this patient share an increase in proteins of apparent MW of 47 to 54 Kd, this common finding strongly suggests that increased amounts of the 47-Kd protein may be linked to diminished ability of the PMN to polymerize actin or stabilize actin in the F-actin state after polymerization.

The precise biochemical basis for this novel PMN defect remains obscure. The fact that the patient’s cells are deficient in chemotactic response to leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and FMLP, yet exhibit normal to increased secretion and respiratory burst in response to FMLP argue against a defect in the FMLP receptor or signal transduction mechanisms common to the actin polymerization and the respiratory burst responses such as G protein, PI<sub>3</sub>, or Ca<sup>2+</sup> flux. The presence of the abnormal amounts of two proteins is suggestive of a role for one or both of these proteins in the defective motility. The function of these two proteins in regulating actin polymerization and the functional link between them is still unknown and further studies will be needed to delineate their relation. However, the reciprocal relationship between decrease in 89-Kd and increase in 47-Kd proteins cannot be explained by selective proteolysis of the 89-Kd protein because (1) the excess of 47-Kd protein is too large relative to the total amount of 89-Kd proteins in normal cells to account for the increase in 47-Kd protein; (2) the cells were treated with DFP before and during cell solubilization to inhibit proteolysis; (3) the abnormalities were consistently observed in numerous (five) different sample preparations; and (4) polyclonal antibod-
ies raised to the crude 47-Kd 1D-SDS-PAGE band reacted only with 47-Kd proteins and not with any protein of higher molecular weight including 89-Kd on immunoblots (data not shown). Therefore, the 47-Kd protein and 89-Kd protein are antigenically distinct. While the 47-Kd protein has not yet been purified, preliminary sequence data on the first five amino acids as well as its pI indicate that it is not analogous to p47 of platelets (Glu-Pro-Lys-Arg-Ile) or p47 of neutrophils (Gly-Asp-Thr-Phe-Ile). In summary, we have described an infant with recurrent infection and a severe defect in neutrophil motile functions. This defect appears to be inherited as a recessive trait and is associated with impaired ability to polymerize actin, an increase in a 47-Kd protein, and a profound decrease in an 89-Kd protein. These studies describe a new, clinically important disorder associated with recurrent infections and draw attention to the proteins of apparent MW 47 Kd and 89 Kd that may be of critical importance in the regulation of the microfilamentous cytoskeleton in hematopoietic cells in humans.

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