Recurrent Severe Infections in a Child With Abnormal Leukocyte Function: Possible Relationship to Increased Microtubule Assembly

By John I. Gallin, Harry L. Malech, Daniel G. Wright, John K. Whisnant, and Charles H. Kirkpatrick

A 7-yr-old girl with a complex disorder of mesenchymal tissues characterized by recurrent life-threatening infections, growth retardation, difficulty in healing, hemangiomas, anemia, thrombocytopenia, and severe leukocyte dysfunction was studied. Detailed studies of her leukocytes revealed previously undescribed ultrastructural and metabolic abnormalities. Centriole-associated microtubule counts in granulocytes prepared under conditions of directed migration were markedly increased (over 300%). Basal mononuclear cell cyclic GMP levels were 400% greater than those observed in cells from age-matched controls. These abnormalities were associated with multiple defects of leukocyte function. Neutrophils were unable to orient in response to a gradient of chemotactic factor. In neutrophils and monocytes both random and directed locomotion of the cells were markedly abnormal when assessed by a $^{51}$Cr radioassay or by morphologic filter techniques. Furthermore, leukocytes showed diminished adherence to nylon wool, delayed bactericidal activity for Staphylococcus aureus and Streptococcus viridans, and subnormal secretion of the granule-associated enzyme lysozyme in response to A23187, phorbol myristate acetate, or adherence to nylon wool surfaces. In addition, both humoral and cellular immune functions of lymphocytes were abnormal. The abnormalities in this patient's leukocytes may have been related to increased microtubule assembly and elevated cGMP. The possibility of a common defect of mesenchymal cells is intriguing but confirmation must await additional studies in other patients with a similar disease.

VARIOUS CLINICAL SYNDROMES characterized by recurrent infections have been associated with abnormalities of leukocyte locomotion, phagocytosis, and bactericidal capacity.$^{1,2}$ Only recently, however, have investigators begun to clarify the structural and biochemical bases for these leukocyte defects. Boxer et al. reported a patient with recurrent infections and well-defined structural and functional defects of leukocytes related to poorly polymerizable actin.$^{3}$ More recently, evidence has been presented that the functional abnormalities in the Chediak-Higashi syndrome, a disorder with defective leukocyte locomotion$^{4}$ and bactericidal activity,$^{5}$ may be related to deficient microtubule assembly and to abnormal cyclic nucleotide metabolism.$^{6,7}$ This communication will describe a patient with recurrent life-threatening infections who had multiple morphologic and functional abnormalities of her leukocytes.
and whose polymorphonuclear leukocytes showed markedly increased microtubule assembly.

CASE REPORT

W.S. was a white female born after term pregnancy complicated by urinary tract infection. Birth weight was 4 lb 14 oz. The parents were not related, and there was no family history of recurrent infection, immunodeficiency, or hematologic disease. Both a female and a male sibling remained normal at ages 7 and 1 yr.

The patient presented at 3 mo of age with digital and oral hemangiomata and recurrent respiratory infections. She was found to have severe thrombocytopenia, lymphopenia, and anemia. Hematologic abnormalities persisted and worsened as hepatosplenomegaly developed. There were multiple pyogenic infections, including pneumococcal sinusitis, Hemophilus influenzae otitis media, and three episodes of Streptococcus pneumoniae lobar pneumonia. In addition, pathogenic staphylococci and streptococci were repeatedly cultured from small areas of cutaneous inflammation. In spite of multiple courses of antibiotics, the recurrent otitis and sinusitis could not be controlled sufficiently to prevent hearing loss and facial maldevelopment. Overall growth and development were delayed out of proportion to chronic infection and anemia, probably because the severe oral scarring that followed the hemangiomatosus-thrombotic episodes was an obstacle to adequate nutrition.

This patient was seen by one of us (J.K.W.) for several years and was admitted to the N.I.H. Clinical Center for further study at age 7 yr. Physical examination revealed a thin, pale child who was alert and active. She had hypertrichosis and severe microstomia with gingivitis. Both liver and spleen were enlarged and firm. Bilateral inspiratory rales and ronchi were heard, and a chest x-ray revealed atelectasis and fibrosis of the right middle lobe, parenchymal scarring of the left lung, and narrowing of the trachea. Peripheral blood counts are summarized in Results. Laboratory analyses at times of no clinical infection showed normal urinalysis, normal BUN and creatinine, no liver dysfunctions, normal serum calcium and phosphorus, and normal 24-hr 17-ketosteroid excretion. Long bone x-rays and marrow aspirates showed no evidence of mucopolysaccharidosis, and epiphyses were normal. Erythrocyte purine salvage pathway enzyme activities (adenosine deaminase and purine nucleoside phosphorylase) were normal. A karyotype from peripheral leukocytes was a normal 46,XX pattern on three occasions. Immunologic and phagocytic dysfunctions are presented under Results. The patient died of disseminated varicella at the age of 8 yr, 8 mo.

MATERIALS AND METHODS

Leukocytes

Leukocytes were obtained from heparinized peripheral blood by the Ficoll-Hypaque technique. Mononuclear cells obtained by this method were washed twice in Hank’s balanced salt solution. After removal of mononuclear cells, the polymorphonuclear leukocytes were separated from erythrocytes by dextran sedimentation and residual erythrocytes were removed by hypotonic saline lysis.

Immunologic Evaluations

Leukocyte counts and differential staining were done by standard methods. The subpopulations of lymphocytes were determined on cells that had been separated on density gradients of Ficoll and Hypaque. T lymphocytes that spontaneously formed rosettes with three or more sheep erythrocytes were enumerated after 1 3 and 24 hr of incubation at 4°C. Immunoglobulin-bearing B lymphocytes were detected with fluorescein-labeled antisera. Cells were examined under both fluorescent light and epillumination to identify monocytes.

Serum levels of IgG, IgM, and IgA were measured by radial immunodiffusion using commercial reagents (Meloy Labs, Springfield, Va.). IgE was measured by a commercial radioimmunoassay (Pharmacia).

Assessment in vivo of cell-mediated immune responses included skin testing with purified protein derivative (PPD), candida, streptokinase-streptodornase, and tetanus toxoid. Cutaneous responses were scored at 15 min and 6, 24, 48, and 72 hr as described previously.
Lymphocyte transformation studies were conducted as described previously, except that the leukocytes were first fractionated through Ficoll-Hypaque density gradients and the cell density in the cultures was reduced to 250,000 lymphocytes/ml. Incubations were conducted in either 10% autologous or pooled human group AB serum. The cells were labeled with tritiated thymidine and harvested as described previously. Mixed leukocyte reactions were done with mitomycin-blocked allogeneic cells as described elsewhere.

**Phagocytic Cell Function**

Leukocyte adherence was measured using a modification of the technique of MacGregor et al. One milliliter of heparinized blood was placed in a 1.0-ml tuberculin syringe that had been packed with 50 mg of nylon wool (Leuko-Pak, Fenwal Laboratories, Morton Grove, Ill.). The blood was allowed to drain through the nylon wool and a 1/2-inch 19-gauge needle into a plastic test tube at room temperature. The total white cell and differential counts on the pre- and post-nylon adherence samples were compared and the percentage of adherent cells determined. Means ± SEM (three determinations) were calculated for each assay.

Neutrophil locomotion was assessed utilizing a radioassay employing $^{51}$Cr-labeled leukocytes and a double micropore filter system with acrylic chemotaxis chambers. With this assay $^{51}$Cr-labeled cells migrated through the upper and into the lower 3.0-μm cellulose nitrate micropore filter (Sartorius, Göttingen, Germany). Chemotaxis was expressed as leukocyte-associated radioactivity in the lower filter (corrected counts/min) as previously described. For some studies of neutrophil chemotaxis a morphologic assay was used in which cells migrated into a 3.0-μm cellulose nitrate filter (Sartorius). After incubation, filters were removed, fixed in methanol, stained in Mayer's hematoxylin, dehydrated in alcohol, and cleared in xylene. Migration of the population of cells was determined by counting the number of cells/high-power field (hpf) at varying depths in the filter as previously described. Five fields were read in each of two filters at each depth and the mean ± SEM determined.

Mononuclear cell locomotion was evaluated by the morphologic assay of Snyderman et al. using 5.0-μm-pore polycarbonate filters (Neuroprobe, Bethesda, Md.) in the same acrylic chemotactic chambers used for neutrophil chemotaxis. Incubation time was 90 min, and chemotaxis was expressed as cells/hpf migrating to the lower surface of the polycarbonate filter.

Spontaneous random migration of cells was assessed using buffer as the stimulus. Stimulated random locomotion was measured by placing equal concentrations of chemotactic factors in both the cell and stimulus chambers so that there was no gradient of chemotactic factor.

Chemotactic stimuli were *Escherichia coli* endotoxin (Difco Laboratories, Detroit, Mich.) activated serum or sodium caseinate (5 mg/ml in 0.85% NaCl, Difco). Measurement of lysozyme in the incubation media of neutrophils after exposure of the cells to phorbol myristate acetate (PMA) (Consolidated Midland, Brewster, N.Y.), ionophore A23187 (courtesy of Eli Lilly, Indianapolis, Ind.), or adherence to nylon wool (Leukopak, Fenwal Laboratories) was done by a standard turbidometric assay. The cytoplasmic enzyme lactate dehydrogenase (LDH) was also assayed by standard techniques, and nitroblue tetrazolium dye reduction by leukocytes was assessed as previously described.

Leukocyte surface charge was determined by measuring the electrophoretic mobility of the cells in the frontal plane of a cytopherometer fitted with platinum electrodes (Carl Zeiss, New York) as described previously.

**Morphologic Analysis of Neutrophils Under Conditions of Directed Migration**

Chemotactic chambers were fitted with 0.45-μm-pore polycarbonate filters (Millipore, Bedford, Mass.). These small-pore size filters permitted pseudopod penetration but did not allow cell migration, and in a gradient of a chemotactic stimulus the cells oriented as a monolayer on the filter surface. With this technique it was possible to evaluate orientation of neutrophil internal structures. Nuclear location was determined as described previously and centriole location relative to the nucleus was determined by serial sections through adjacent cells on the filters. Centriole-associated microtubules were counted as previously described, where microtubules were defined as structures that had straight, parallel sides 240 280 Å apart, were at least 550 Å long, and were more electron dense than ground cytoplasm.
Cyclic Nucleotide Assays

Mononuclear leukocytes obtained by Ficoll-Hypaque density gradients were washed and suspended at a density of $5 \times 10^6$ mononuclear cells (approximately $10^5$ monocytes, 70% lymphocytes, and 90% granulocytes) in 2 ml of Hank's balanced salt solution. For certain studies cells were exposed to 100 $\mu$M serotonin for 5 min. In all experiments the reactions were stopped with cold perchloric acid and the cyclic nucleotides were extracted and separated as described previously. Cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) concentrations were determined by radioimmunoassays using commercial reagents (Collaborative Research, Waltham, Mass.). The results were expressed as pmols of cyclic nucleotide per $10^7$ cells.

RESULTS

Throughout the period of observation, the patient had persistent, mild, hypochromic anemia with subnormal serum iron saturation that was resistant to iron therapy. The peripheral leukocyte counts were usually $5-10 \times 10^9$ cells/liter, and typical differential counts showed about 65% segmented neutrophils, 5% band forms, 10% lymphocytes, 15% monocytes, and 5% eosinophils. The peripheral smear contained dyspoietic mature granulocytes, marked anisopoikilocytosis, and hypochromia. The platelet counts were $5-25 \times 10^9$/liter. Survival of transfused platelets was normal. The bone marrow was markedly hypercellular and showed granulocytic hyperplasia, eosinophilia, mild plasma-cytosis, and mild megaloblastic changes in the erythroid series. Megakaryocytes were increased in number and showed nuclear immaturity and increased basophilia. It was concluded that the chronic thrombocytopenia was the result of impaired platelet maturation rather than a consumptive process or an intrinsic platelet defect. The bone marrow contained no stainable iron. Stool guiacs were consistently negative. The anemia was considered to be iron deficiency anemia, possibly partially secondary to bleeding and nutritional deficiency, although the anemia responded poorly to supplemental iron.

Studies of Cell Locomotion

Neutrophil and mononuclear cell random and directed locomotion were markedly impaired. As shown in Table 1, according to the radioassay of neutro-

<table>
<thead>
<tr>
<th>Assay</th>
<th>Neutrophils in Lower Filter* (car cpm)</th>
<th>Mononuclear Migration† (Cells/hpf)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Patient</td>
</tr>
<tr>
<td>Random migration buffer</td>
<td>330 ± 45(6)</td>
<td>95 ± 18(6)</td>
</tr>
<tr>
<td>Directed migration E. coli endotoxin-activated sera (5%)</td>
<td>2201 ± 243(8)</td>
<td>178 ± 63(8)</td>
</tr>
<tr>
<td>Caseinate (5 mg/ml)</td>
<td>3067 ± 120(2)</td>
<td>278 ± 30(2)</td>
</tr>
</tbody>
</table>

*Neutrophil migration using a $^{51}$Cr radioassay (see Materials and Methods).
†Mononuclear cell migration using a morphologic assay (see Materials and Methods).
§Number of separate experiments in parentheses.
$\dagger$Significance of difference from normal, Student's $t$ test.
phil locomotion the ability of $^{31}$Cr-labeled leukocytes to migrate through the upper filter and into the lower filter was significantly decreased. This finding was true for both random migration (buffer stimulus) and directed migration (endotoxin-activated serum or sodium caseinate as stimuli). These differences were not due to differences in the uptake or retention of $^{31}$Cr in the normal or patient’s cells. In related studies measuring stimulated random migration, in which cells were exposed to a uniform concentration of the chemoattractant (endotoxin-activated serum), there was no enhancement of random migration of the patient’s cells as was noted for normals (data not shown). The distribution of the patient’s neutrophils migrating into a cellulose nitrate filter under conditions of directed migration was also measured, and in contrast to normal cells very few of the patient’s neutrophils penetrated the filter matrix, even when incubated for 3 hr (data not shown).

Monocyte locomotion was also measured; as shown in Table 1, a severe defect was present. The response of the patient’s cells was about 10% of normal ($p < 0.01$), and the defective monocyte locomotion was confirmed independently by Dr. Ralph Snyderman of Duke University Medical Center.

The patient’s serum generated normal chemotactic activity when activated with endotoxin. Evaluation of complement components (courtesy Dr. M. Frank) revealed normal $CH_2$ activity and no deficiency of the third or fourth complement components. In addition, there was no evidence that her serum had a cell-directed inhibitor of leukocyte locomotion. Incubation of normal cells for 30 min in the patient’s serum (10%) did not affect their locomotion in a manner that differed from normal serum.

Associated with the defect of neutrophil and monocyte locomotion was abnormal adherence to nylon wool. In studies with leukocytes from normal subjects $75_{\pm}^\%$ of neutrophils and $79_{\pm}^\%$ of monocytes adhered to nylon wool. In contrast, studies with the patient’s cells showed that only $37_{\pm}^\%$ of her neutrophils ($p < 0.01$ versus normal) and $50_{\pm}^\%$ of her monocytes ($p < 0.05$ versus normal) adhered to the nylon wool column.

When the neutrophils and monocytes were examined with a light microscope while they were moving on a glass slide, they did not spread normally and the neutrophils sent out very few pseudopods.

Bactericidal and Secretory Activity

The bactericidal capacity of the patient’s neutrophils was also abnormal. Whereas normal neutrophils killed $75_{\pm}^\%, 90_{\pm}^\%,$ and $98_{\pm}^\%$ of ingested $S. aureus$ at 20, 45, and 90 min, the patient’s neutrophils killed only $40_{\pm}^\%, 79_{\pm}^\%,$ and $86_{\pm}^\%$ of the organisms at the same times ($p < 0.01$ at 20 min, $p < 0.05$ at 45 min). A similar bactericidal defect was apparent using $S. viridans$ as the test organism. The delay in killing by the patient’s cells suggested abnormal ingestion but a normal biochemical bactericidal mechanism. In a single experiment phagocytosis of $^{14}$C-labeled $S. aureus$ was less than $50_{\pm}^\%$ of normal ($p < 0.05$). Resting and stimulated NBT tests were normal. Secretion of lysozyme in response to nonphagocytic degranulating stimuli was also abnormal (Fig. 1). Although the total neutrophil lysozyme level was normal, secretion of the enzyme in response to PMA, A23187, and nylon wool was $52_{\pm}^\%, 53_{\pm}^\%,$ and $36_{\pm}^\%$ of the respective
mean normal values. The patient’s sister’s cells secreted lysozyme normally. Under these experimental conditions, lactate dehydrogenase was not released by normal cells or by the patient’s cells.

**Orientation of Neutrophils in Response to a Gradient of Chemotactic Factor**

As shown in the low-power electron micrographs in Fig. 2, the patient’s cells had a bizarre morphology when compared to normal. The cytoplasmic membranes had blebs that often contained portions of nuclei, and there was abnormal cellular orientation. With normal cells, the pseudopodia extended into the filter, and the nuclei were situated towards the trailing edge of the cell, away from the filter. In contrast, the cells from the patient showed no preferential alignment or orientation along the filter. These findings were quantitated as described in Materials and Methods and Ref. 19. Whereas in normal cells the nuclei are located towards the back of the cell, the nuclei in the patient’s cells had a random distribution ($p < 0.001$ compared to normals).

From studies of serial sections of cells responding to chemotactic stimuli, we previously showed that the centriole, which appears to be the organizing center of the microtubules, is located between the nucleus and the advancing pseudopods. A similar analysis with cells from our patient disclosed essentially random positioning of the centrioles (Table 2). Another striking abnormality was the increased number of microtubules visualized in the patient’s cells and these were present as dense bundles. An example of the bundles of microtubules is shown in Fig. 3. Normal cells did not exhibit such extensive parallel arrays of multiple microtubules.

Centriole-associated microtubules visible by electron microscopy were quantitated as previously described. The quantitative analysis of microtubules is shown in Fig. 4 and indicates significantly increased numbers of visualized microtubules. There were no obvious discernable morphologic abnormalities of the microfilaments with a normal loose meshwork of the submembranous filaments in the leading edge of the cells.

**Immunologic Studies**

After 1–3 hr of incubation at 4°C, 13%, 18%, of the patient’s blood lymphocytes formed rosettes with sheep erythrocytes (E rosettes), while the normal
controls had 47°, 62°, rosette-forming cells. After 24 hr of incubation, only 6°, 7°, of the patient’s lymphocytes formed E rosettes, while control values were 55°, 70°.

This apparent loss of rosette-forming capacity by lymphocytes during incubation at 4°C was not due to cell death. When the patient’s lymphocytes were in-
Table 2. Neutrophil Centriole Orientation During Conditions of Chemotaxis

<table>
<thead>
<tr>
<th>Cells</th>
<th>Number of Cells in Which Centriole Was Identified</th>
<th>Position of Centriole Relative to Nucleus (Number at Each Location)</th>
<th>Mean Score*</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td></td>
<td>Above</td>
<td>Intermediate</td>
<td>Below</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>0</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Patient</td>
<td>10</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

*Mean score: number of centrioles above nucleus - number of centrioles below nucleus/total number of centrioles, ± standard error.
†Significance level of difference from a mean score of 0, consistent with a random distribution of centrioles.

Fig. 3. High-magnification electron micrograph of a portion of one of the patient’s neutrophils on a 0.45-μm cellulose nitrate filter under conditions of directed migration with E. coli endotoxin-activated serum as the chemoattractant. Note the bundles of microtubules (mt). Bar, 0.25 μm. ×32,000.
cubated with sheep erythrocytes in the presence of 8-bromo-cGMP (10^{-6} M), there was no reduction in the percentage of rosette-forming cells between 1–3 and 24 hr. This procedure had no effect on rosette formation by normal lymphocytes. In two experiments, addition of thymosin fraction V (200 µg/ml) to the suspensions of lymphocytes and sheep erythrocytes produced significant increases in the percentage of rosette-forming cells (thymosin-treated: 31.33% ± 3.33%, control 16.00% ± 2.00%, p = 0.02). Thymosin did not affect rosette formation by normal cells. Only 3% of the patient’s lymphocytes had surface immunoglobulins, unlike control cells, of which 10%–20% had surface immunoglobulin. Thus approximately 80% of the patient’s peripheral blood lymphocytes lacked the membrane properties that characterize B and T cells.

Serum concentrations of IgG, IgM, and IgE were normal (5.8 mg, 0.88 mg, and 68 IU/ml, respectively), while IgA was always elevated over normal but varied between 400 and 2000 mg/dl. There was a normal antibody response to immunization with tetanus toxoid, but the levels did not persist.

Numerous abnormalities of cell-mediated immunity were apparent. All skin tests for delayed hypersensitivity to environmental antigens were negative, and these antigens did not stimulate lymphocyte transformation in vitro. Lymphocyte responses in vitro to mitogens were also subnormal (Table 3), and these abnormalities in lymphocyte function could not be corrected by culturing the patient’s cells in serum from normal donors.

In the mixed leukocyte reaction, the patient’s cells were markedly responsive to mitomycin-blocked cells from her sister and her mother (Table 3). However, the patient’s cells were incapable of stimulating DNA synthesis in allogeneic cells. Indeed, addition of mitomycin-blocked cells from the patient produced responses that were less than those of the autologous controls. On two attempts, HLA-A and HLA-B antigens could not be detected on the patient’s leukocytes by a standard cytotoxicity assay.
Table 3. Responses In Vitro of Lymphocytes to Mitogens and Allogeneic Cells

<table>
<thead>
<tr>
<th>Mitogens</th>
<th>cpm</th>
<th>Patient</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>4,072</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PHA (1 μg/ml)</td>
<td>189,881</td>
<td>44</td>
<td>100-700</td>
</tr>
<tr>
<td>Concanavalin A (20 μg/ml)</td>
<td>52,936</td>
<td>13</td>
<td>60-200</td>
</tr>
<tr>
<td>Pokeweed Mitogen (0.1 mg/ml)</td>
<td>36,540</td>
<td>9</td>
<td>40-150</td>
</tr>
<tr>
<td>Mixed leukemia reactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient with self</td>
<td>222</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>with sister</td>
<td>44,361</td>
<td>200</td>
<td>—</td>
</tr>
<tr>
<td>with mother</td>
<td>54,127</td>
<td>244</td>
<td>—</td>
</tr>
<tr>
<td>Sister with self</td>
<td>19,162</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>with patient</td>
<td>196</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>with mother</td>
<td>534,000</td>
<td>28.00</td>
<td>—</td>
</tr>
<tr>
<td>Mother with self</td>
<td>4,540</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>with patient</td>
<td>216</td>
<td>0.05</td>
<td>—</td>
</tr>
<tr>
<td>with sister</td>
<td>37,400</td>
<td>8.20</td>
<td>—</td>
</tr>
</tbody>
</table>

Stimulation Ratio = Mean cpm in stimulated cultures divided by mean cpm in unstimulated cultures.

Cyclic Nucleotide Accumulation

The effects of serotonin on accumulation of cyclic guanosine monophosphate by blood mononuclear cells are shown in Fig. 5. Cells from six age-matched control subjects had a mean cGMP level of 4.48 ± 0.65 pmols per 5 × 10⁶ cells under resting conditions; this increased to 8.22 ± 0.16 pmols per 5 × 10⁶ cells after 5 min of incubation with serotonin (p < 0.05). In contrast, the resting cells from the patient contained 16.15 ± 2.45 pmols cGMP per 5 × 10⁶ cells (p < 0.001 versus resting normal cells), and incubation with serotonin produced no changes.

There were no differences in the cAMP content of the patient’s or control mononuclear cells, and none of the agonists studied caused changes in cellular cAMP. We were unable to obtain enough cells to measure cyclic nucleotides in granulocytes.

Leukocyte Membrane Studies

The diminished adherance, locomotion, phagocytosis, and degranulation of leukocytes as well as the inability of lymphocytes to form stable E rosettes and the failure of the patient’s cells to stimulate in mixed leukocyte reactions suggested an abnormality of the cytoplasmic membrane. An abnormality of this severity could be associated with altered cell surface charge. However, in three experiments in which 20 cells were studied in each, the net surface charge (electrophoretic mobility) of the patient’s neutrophils was normal (mean surface charge of 1.83 ± 0.04 μm/sec/V/cm for the patient, 1.76 ± 0.03 μm/sec/V/cm for controls).

Attempts to Correct the Abnormal Leukocyte Locomotion

Attempts were made to correct the abnormal leukocyte locomotion of the patient’s cells in vitro using agents that previously had been shown to affect...
cyclic nucleotide metabolism or microtubule polymerization in normal cells. Prostaglandin E₁ (14 µM), which increases leukocyte cAMP,²² was preincubated with the patient’s cells for 2–3 min; such treatment did not affect the function of her cells. Treatment of her leukocytes with colchicine (10⁻⁸–10⁻⁵ M), concentrations that prevent microtubule polymerization in normal leukocytes,¹⁹ did not affect her leukocyte locomotion. Levamisole (10⁻⁹–10⁻¹ M), which has been shown to increase cGMP and to improve the abnormal chemotactic response of leukocytes from patients with elevated IgE and recurrent staphylococcal skin infections,²³ did not improve the chemotactic responses of this patient’s cells.

DISCUSSION

This patient had a complex array of disorders that may have been related to a common abnormality of mesenchymal tissues. Bone marrow elements including leukocytes, erythrocytes, and megakaryocytes were abnormal. Clinically the patient had abnormal healing suggesting a defect of fibroblast function, and cultures of her fibroblasts in vitro were difficult to establish because the cells divided slowly and adhered poorly to the culture plates. In addition, the recurrent hemangiomata provided clinical evidence for an endothelial cell defect, although these cells were not studied in vitro.

The recurrent life-threatening infections were associated with a complex ar-
ray of abnormal leukocyte functions. The patient’s neutrophils and monocytes were found to be virtually unresponsive to chemotactic stimuli, and the severity of this defect was especially striking when compared to two other disorders in which recurrent infections and deficient leukocyte chemotaxis coexist, the Chediak-Higashi syndrome\(^4\) and the hyper-IgE syndrome.\(^{23,24}\) In the Chediak-Higashi syndrome, chemotactic responses by neutrophils and monocytes are typically 25\(^\circ\) of normal, respectively,\(^{23}\) and in the hyper-IgE syndrome responses by neutrophils and monocytes are usually 50\(^\circ\) and 60\(^\circ\) of normal.\(^{23}\) In contrast, in this patient the neutrophil and monocyte chemotactic responses were 10\(^\circ\) of normal or less. In addition, this patient’s cells had subnormal bactericidal activity, which was probably the result of both impaired phagocytosis and abnormal degranulation. The extracellular secretion of lysozyme by leukocytes in response to degranulating stimuli was also significantly less than normal. Studies of nitroblue tetrazolium reduction, a screening test of oxygen metabolism by leukocytes, was normal.

The patient also had profound abnormalities of lymphocyte function. The numbers of immunoglobulin-bearing and E rosette forming cells were subnormal, and the percentage of lymphocytes that formed E-rosettes at 1-3 hr was greater than that at 24 hr, suggesting that the T lymphocyte membrane receptors for the sheep erythrocytes were unstable or of low affinity. Both cyclic GMP and thymosin have been reported to enhance rosette formation by human T lymphocytes,\(^{25,26}\) but the mechanisms are unknown. Thymosin may promote maturation of “null” cells into T cells.\(^{27}\) Addition of cGMP to our patient’s cells prevented the reduction in the percentage of rosette-forming cells between 1-3 and 24 hr, and thymosin actually increased the percentage of rosette-forming cells. In addition, the cells were poorly responsive to the T and B cell mitogens; it was not possible to determine whether this was because of the small percentage of T and B cells in the blood or indicative of defective interaction between the lectins and the receptors on the cell membrane. There were no responses of lymphocytes to environmental antigens that most subjects have encountered by age 8 yr.

The elevated serum IgA was also of interest because an association of IgA elevation and chemotaxis inhibition has been reported by Van Epps and Williams,\(^{28}\) who postulated that IgA M components might mediate chemotaxis inhibition by binding to the leukocyte surface. However, there was no evidence of IgA myeloma or paraproteinemia in our patient, and we were unable to demonstrate a cell-directed inhibitor of chemotaxis in the patient’s serum.

It seems likely that these defects predisposed the child to repeated infections with a variety of organisms. While most patients with leukocyte chemotactic defects are susceptible to infections with pyogenic bacteria, patients with abnormalities of cell-mediated immunity are susceptible to infections with fungi and viruses. This patient, like patients with other syndromes of “combined” immunodeficiency (Wiskott-Aldrich syndrome, severe combined immunodeficiency, and certain patients with hyper-IgE syndrome) suffered from both recurrent bacterial and viral infections, and her terminal illness was varicella pneumonia.

Recently it was reported that the lymphocytes from three patients with combined immunodeficiency contained parallel tubular arrays.\(^{29}\) These tubules had
a mean diameter of 41 μm and were larger than the microtubules in the leukocytes of our patient. Moreover, when our patient’s cells were examined in serial section, the microtubules originated in the centriole and radiated toward the cytoplasmic membrane.

Our studies did not define a single pathogenic process that would account for the diverse abnormalities of leukocyte function. It was unlikely that the observed abnormalities were due to an acquired viral infection, since on multiple occasions we were unable to culture viruses from her fibroblasts or leukocytes even when she had varicella pneumonia (cultures kindly performed by Dr. Raphael Dolin). Certain data, such as the poor adherence of her cells to nylon wool, the instability of the association between her T lymphocytes and sheep erythrocytes, the failure of her cells to stimulate allogeneic cells in the mixed leukocyte reaction, and our failure to detect HLA antigens on her cells, were compatible with abnormalities of the cell membrane. Impaired chemotactic responses could occur if her cells lacked the receptors for chemotactic signals or were unable to integrate the intracellular mechanisms for directed cell migration.

On the other hand, the functional abnormalities of our patient’s cells may have been due to disturbances in intracellular metabolic events as the primary disorder or as a consequence of membrane abnormalities. For example, the functional events that were abnormal in our patient’s neutrophils, monocytes, and lymphocytes have been associated with cyclic nucleotide metabolism and normal function of the tubulin-microtubule system. Elevation of cGMP is associated with enhancement of leukocyte chemotaxis and degranulation. Microtubule assembly, thought to be controlled by cyclic nucleotides, is currently believed to stabilize neutrophils during chemotaxis and organize granules during secretion. Leukocyte locomotion, however, appears to require a normal tubulin polymerization and depolymerization. If tubulin polymerization is prevented by colchicine or is abnormal, as reported in certain patients with Chediak-Higashi syndrome, chemotaxis is depressed. In contrast, there is recent evidence using immunofluorescent and 3H-colchicine-binding techniques that inhibition of macrophage locomotion by migration inhibitory factor acts via large (150–200) increases in the tubulin polymer and associated elevations of cGMP. Similarly, the neutrophil dysfunction in our patient may have been due to excessive microtubule assembly.

Although our patient died before the potential relationship between microtubule assembly and leukocyte dysfunction could be proved as cause and effect, this possibility clearly exists. The implication suggested by the observations in this patient are of sufficient interest to justify similar studies in other patients to clarify these possibilities.

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