An unusual ultrastructural neutrophil abnormality of unknown function[1]

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An unusual ultrastructural abnormality of neutrophils is reported in two patients who had bronchogenic carcinoma and idiopathic hypereosinophilic syndrome, respectively. The abnormal neutrophils contained numerous elongated nuclear and cytoplasmic inclusions measuring up to 3 by 0.1 μm. Although the chemical nature, etiology, and functional significance of these inclusions are unknown, they may represent an acquired and reversible neutrophil disorder. In contrast to earlier reported cases, neither patient had infectious complications when the abnormality was found.

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In 1975 Linsk et al[1] reported a new ultrastructural abnormality of neutrophils in three members of a family affected by a peculiar primary hematologic disorder. In 1976 we described the same abnormality in two renal allograft recipients.[2,3] Herewith, we report the same abnormality in two patients who had bronchogenic carcinoma and idiopathic hypereosinophilic syndrome, respectively. In addition, we will review the literature on this subject.

Case reports

Case 1. A 52-year-old white male was referred to The Cleveland Clinic Foundation for evaluation of weight loss, progressive dyspnea on exertion, weakness, and an abnormal chest radiograph that showed a left hilar mass. Three months earlier he had been treated for a pulmonary embolism, and he also had a history of angina pectoris and cardiac dysrhythmia. The patient had no history of any known primary blood disorder. Three days after admission, bronchoscopic examination revealed a fungating friable tumor mass in the left mainstem bronchus. A bronchial washing sample contained atypical cells compatible with malignancy.

One week after admission, a generalized papulovesicular rash developed that was diagnosed as drug eruption and treated successfully with Westcort (hydrocortisone valerate) 0.2% cream. The red blood cell count was 5.06×10^9/L; hemoglobin, 15.5 g/dL; hematocrit, 45.8%; and platelets, 299×10^9/L. The white blood cell count was 11.1×10^9/L with 63% neutrophils, 3% band cells, 24% lymphocytes, 6% monocytes, and 4% eosinophils.

Twelve days after admission, a second bronchoscopic examination was performed. A biopsy specimen from the lesion showed severe squamous dysplasia but no definitive evidence of invasive carcinoma, and a bronchial washing specimen also contained atypical cells compatible with ma-

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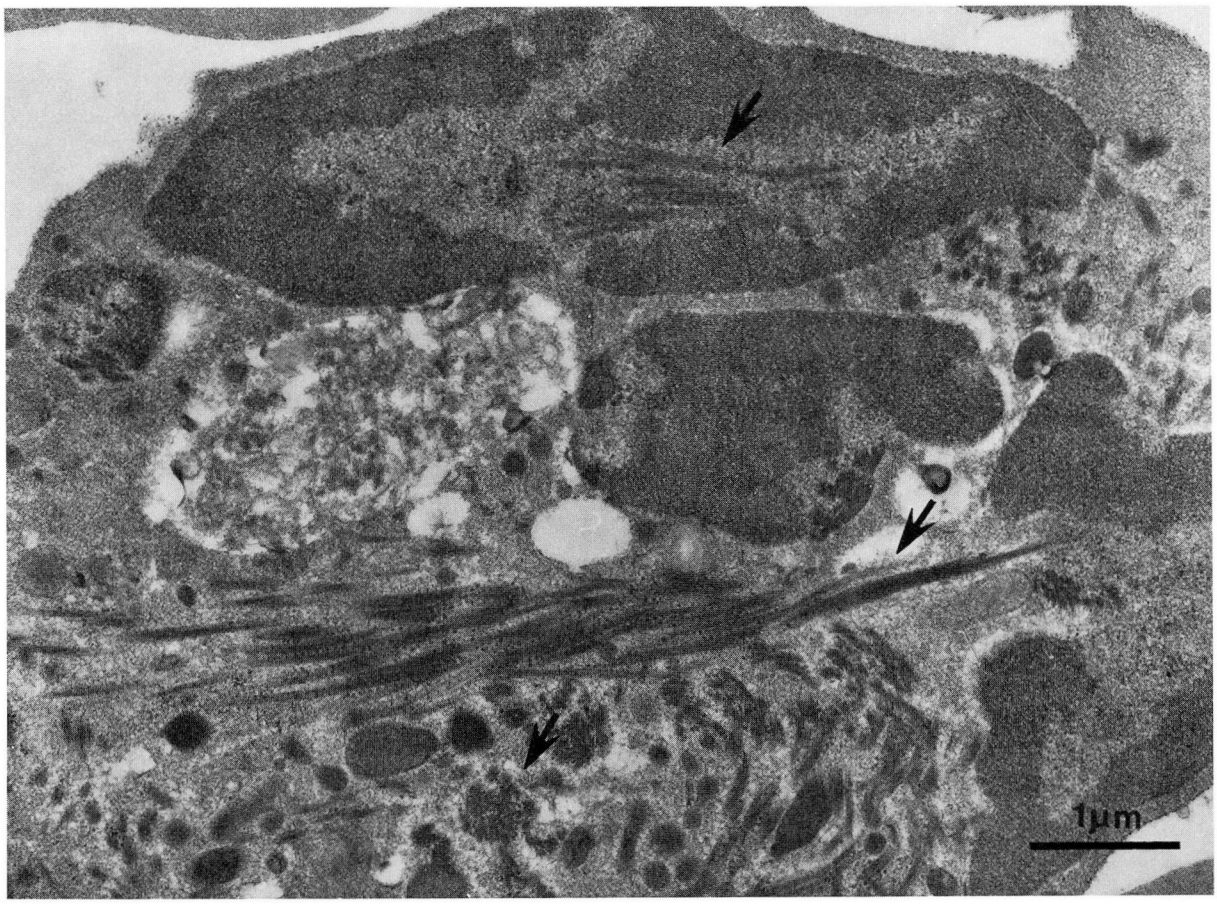

Fig. 1. Case 1. Nuclear and cytoplasmic fibrillary inclusions in a neutrophil of a bronchial washing specimen. A mixture of longitudinal (top arrow), oblique (middle arrow), and cross-sectioned (bottom arrow) inclusions is seen. Uranyl acetate and lead citrate × 20,000.

lignancy. Electron microscopic examination of the latter specimen showed the abnormal neutrophils (*Fig. 1*). Mediastinoscopy and left thoracoscopy, performed five days later, revealed tumor invasion into the hilar structures and pericardium. Histopathologic examination of the tumor showed poorly differentiated squamous-cell carcinoma.

The lesion was considered inoperable and arrangements were made for the patient to receive radiation therapy in a hospital near his home. The patient had not received any recent blood transfusion and had no infections or fever during the entire hospitalization period. The patient responded well to radiation therapy but repeated episodes of hemoptysis developed several months later. Follow-up examination one year later showed opacification of the left lung, probably secondary to radiation therapy, and probable tumor recurrence on the scalp. Again no history of infections was found. We did not have the opportunity to perform additional electron microscopic studies.

Case 2. A 16-year-old white boy was first admitted to Sutter Memorial Hospital in November 1975 for evaluation of anemia and marked eosinophilia. Seven months before, during an office examination for a painful hip, his physician had noted a white blood cell count of 22.0×10^9/L with 58% eosinophils.

When first seen at Sutter Memorial Hospital, the patient was asymptomatic except for a one-week history of cough productive of yellow sputum. There was no history of asthma, wheezing, allergic rhinitis, abdominal pain, nausea, vomiting, diarrhea, jaundice, travel outside the United States, or prior illness. Physical examination was unremarkable except for a Grade 3/6 holosystolic murmur, several shotty 0.5-cm lymph nodes in both axillary and inguinal regions, a spleen tip felt at 1 cm below the left costal margin, and fine rales in both lower lung fields. His chest radiograph showed bilateral lower lobe infiltrates and laboratory findings were: hemoglobin, 9.4 g/dL; hematocrit, 0.30%; platelet count, 63.0×10^9/L; white cell count, 28.0×10^9/L with 70% eosinophils, 12% segmented neutrophils, 18% lymphocytes, and no immature forms seen. Electrolytes and liver and kidney function tests were normal. Serum immunoglobulin levels were normal: IgG, 1,680 mg/dL; IgA, 345 mg/dL; IgM, 228 mg/dL. Skin tests for tuberculosis, coccidioidomycosis, and candida, as well as complement fixation test for coccidioidomycosis, were negative. Sputum and bronchoscopic examination did not reveal any evidence of parasitic disease and eosinophilic tissue infiltration was not seen on the bronchial biopsy specimen. Stool examinations for ova and parasites were repeatedly negative and radio-

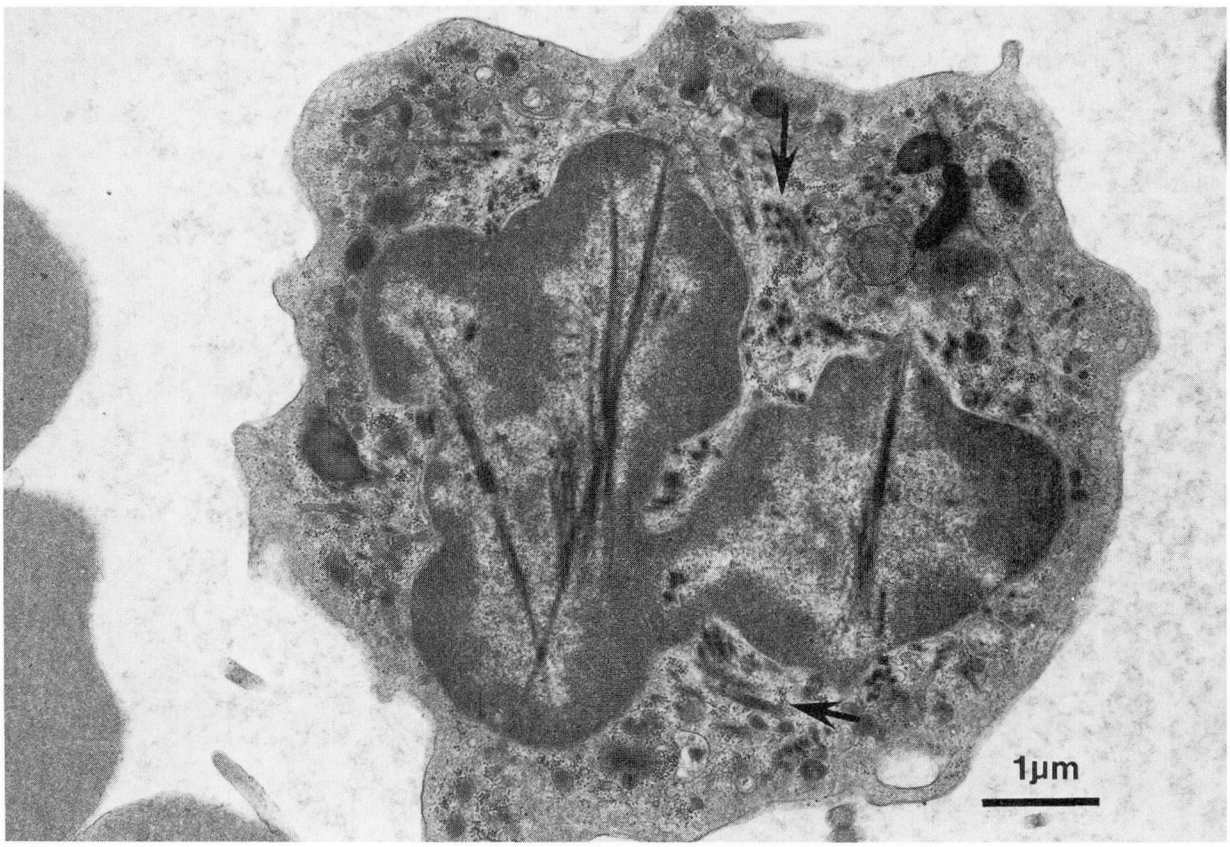

Fig. 2. Case 2. Nuclear and cytoplasmic fibrillary inclusions in a peripheral blood neutrophil. There is a predominance of longitudinally sectioned inclusions in the nucleus and a predominance of cross-sectioned (arrows) inclusions in the cytoplasm. Uranyl acetate and lead citrate × 17,100.

logic examination of the stomach, small bowel, and colon were unremarkable. Bone marrow aspiration and biopsy revealed hypercellularity with marked eosinophilia but normal maturation of erythroid, myeloid, and megakaryocytic series. A diagnosis of idiopathic hypereosinophilic syndrome was made. Daily treatment with oral digoxin, 0.25 mg; furosemide (Lasix), 80 mg; and prednisone, 60 mg, was begun on November 22, 1975.

The patient had a brief symptomatic response. Peripheral blood obtained on January 7, 1976, revealed a total leukocyte count of 38.3×10^9/L with 41% segmented neutrophils, 10% band cells, 15% lymphocytes, 3% monocytes, 23% eosinophils, 4% metamyelocytes, and 2% myelocytes. Electron microscopic examination of this specimen showed the abnormal neutrophils (*Figs. 2* and *3*). Right and left-sided heart catheterization on January 8, 1976, revealed severe mitral insufficiency. The hemoglobin level was 8.1 g/dL, white cell count 44.0×10^9/L with 52% eosinophils, platelet count 76.0×10^9/L, and the spleen was now felt 4 cm below the costal margin. Vincristine (Oncovin) 2.0 mg IV weekly was begun on January 9, 1976, prednisone was resumed at 60 mg daily for 2 weeks before reduction to 60 mg on alternate days, and digoxin and furosemide were continued at previous dosage levels. Progressively severe mitral regurgitation necessitated mitral valve replacement and insertion of a Hancock valve on April 1, 1976. The spleen and splenic hilar lymph node were also removed at surgery. The spleen weighed 582 g and revealed marked eosinophilia with areas of extramedullary hematopoiesis and no reactive follicles. The lymph node also revealed eosinophilia with no reactive follicles.

The remainder of the clinical course was characterized by progressively severe and intractable heart failure, intermittent occurrence of bilateral pulmonary infiltrates and effusions, recurrent anemia, and thrombocytopenia with leukocytosis and eosinophilia. In March 1977, an open lung biopsy revealed interstitial pneumonitis with prominent eosinophilia and hemosiderosis. In December 1977, he died of progressive intractable heart failure with bilateral pulmonary infiltrates. At the time of his death, the hemoglobin level was 7.6 g/dL, white cell count 127.0×10^9/L, and platelet count 47.0×10^9/L. The eosinophils were 58% of the white cell differential count and occasional blast cells and nucleated red cells were also noted. An autopsy was not performed.

Electron microscopy study

A bronchial washing fluid sample was obtained from the first patient and a heparinized venous blood sample was drawn from the second patient. The samples were centrifuged at 700 rpm for 10

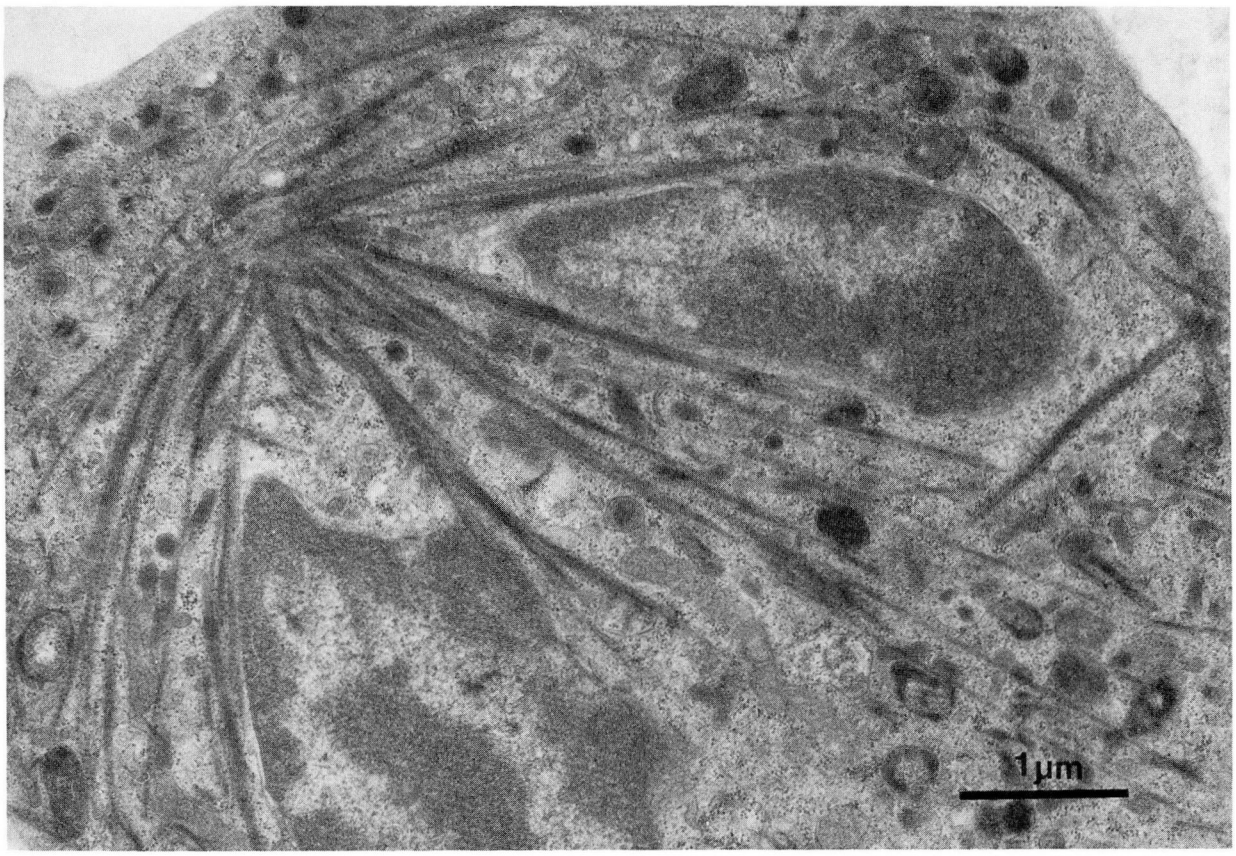

Fig. 3. Case 2. Cytoplasmic fibrillary inclusions in another peripheral blood neutrophil. In this sectional plane, the inclusions tend to converge in one pole of the cell. Uranyl acetate and lead citrate × 23,600.

minutes and the supernatant discarded. Cold 3.75% glutaraldehyde solution,[4] buffered with sodium cacodylate containing sucrose (pH 7.4), was gently poured into the tube and the cell pellet was fixed for two hours at 4° C. After fixation the specimen was trimmed into 1-mm cubes. The cubes were then rinsed in the same buffer, post-fixed for one hour in 1% osmium tetroxide[5] in phosphate buffer containing glucose, dehydrated in an ascending series of ethanol solutions, and embedded in epoxy resin according to the method of Spurr.[6] Sections 1 µm thick were stained with Paragon multiple stain[7] and examined by light microscopy. Ultrathin sections of selected blocks were mounted on 200-mesh copper grids, stained with 1% uranyl acetate[8] followed by lead citrate,[9] and examined with a transmission electron microscope.

Results

The abnormal neutrophils contained numerous elongated inclusions (*Figs. 1–3*). They were present in most of the cytoplasm except in the pseudopods, and to a much lesser extent in the nuclear interchromatin areas. These cytoplasmic inclusions were multidirectional since longitudinal, oblique, and cross-sectioned profiles were observed in the same sectional plane. They measured up to 3 µm by 0.1 µm in greatest dimensions; they were not membrane-bound and they seemed to consist of tightly packed microfibrils without any definite periodic banding. Individual microfibrils could not be reliably characterized or measured. In some areas the possible fibrillary substructure was replaced by a homogenous electron-dense material but a crystalline arrangement (lattice) was never seen. Hollow centers were not definitively detected in the fibers. In some cytoplasmic areas, electron-dense spherical granules (probably glycogen) were seen in close proximity to the fibers. The inclusions were not found in lung epithelial cells of the first case, or in other peripheral blood leukocytes of the second case. The Wright-Giemsa-stained peripheral smear ob-

tained from the second patient on January 7, 1976 did not reveal the abnormal inclusions that were identified by electron microscopic examination of the same specimen.

Discussion and review of the literature

This report describes two additional cases of a rare ultrastructural abnormality of neutrophils in two unrelated patients who had bronchogenic carcinoma and idiopathic hypereosinophilic syndrome, respectively. In 1976 we described the same fibrillary inclusions in tissue neutrophils of two unrelated patients, age 35 and 42 years, respectively. These patients had received renal allografts because of chronic renal failure secondary to hereditary nephritis (Alport's syndrome) and arteriolar nephrosclerosis, respectively. Neither patient was affected by any known primary blood disorder. We suggested that these inclusions represented an acquired abnormality of the neutrophils, since two previous specimens from the first patient had not shown the inclusions.[2,3] Linsk et al[1] in 1975 had reported neutrophils containing inclusions of a similar structure, although they were described as "crystalloid," in peripheral blood of three members of a family affected by a peculiar primary hematologic disorder characterized by myeloid, erythroid, and immunologic defects. They thought that this ultrastructural finding in three out of six siblings was highly suggestive of a kindred-linked abnormality, and that they might be dealing with a new stem-cell disorder. Dobbins[10] reported that these "crystalline" inclusions might not be characteristic of this stem-cell disorder since he had found them in neutrophils infiltrating colonic tissue of a patient with Crohn's disease. In a subsequent short communication, Linsk et al[11] confirmed that the morphologic abnormality of our cases was identical to that seen in their cases.

In 1978 Nanba et al[12] reported the same fibrillary inclusions in peripheral blood neutrophils of a patient with myelofibrosis and also in one of his daughters. Electron-microscopic studies of the leukocytes of three siblings and two other daughters did not show the abnormal inclusions. Two years later, Tatsumi and Wada[13] described inclusions of similar structure in peripheral blood leukocytes of a patient with Hodgkin's granuloma. They attributed the development of this abnormality to treatment with vinblastine. However, none of our five patients studied to date was receiving vinblastine when the inclusions were detected. These inclusions are also structurally different from those cytoplasmic microtubular crystals and ribosomal complexes experimentally induced by periwinkle alkaloids (vinblastine and vincristine sulfate).[14,15] In 1981, we reported the same ultrastructural abnormality in peripheral blood neutrophils of an additional kidney transplant recipient who was still alive and had a functioning renal allograft one year later. The inclusions were not present in other circulating leukocytes. Two previous blood samples obtained from this patient two and three weeks earlier showed normal neutrophils, as did a specimen obtained two weeks later.[16] This further indicates that these fibrillary inclusions represent an acquired and reversible abnormality that involves exclusively the neutrophilic granulocytes; however, their chemical nature and functional significance are not known. Their ultrastructural appearance indicates that they probably derive from endogenous material, most likely cytoskeletal proteins. It is unlikely that this material consists of an exogenous fibrillary protein such as fibrin, since it is present in nuclei and absent in phagosomes.

Some of the previously reported patients with this abnormality had infections. However, neither of the two patients reported here had any history of infection when the abnormal neutrophils were identified. Since this disorder is reversible and possibly of short duration, it may not manifest itself clinically in an otherwise immunologically uncompromised patient. Thus, only functional studies with affected neutrophils would help to elucidate the physiological significance of this morphologic abnormality.

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