The Chediak-Higashi Anomaly

A Report of Two Cases

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This rare anomaly is characterized by abnormal granules in the leucocytes and was first described by Béguez César (1943). Further reports by Chediak (1952) and Higashi (1954), which emphasized the haematological features, led Sato (1955) to associate their names with the anomaly. The abnormal cytoplasmic granules in the neutrophil polymorphs are large, up to 4μ in diameter, and stain a greenish-grey colour with Romanowsky stains; they are peroxidase positive and stain with Sudan black B but not with periodic acid-Schiff stain (PAS). In the eosinophils and basophils the granules appear normal in shape and colour, but they are excessively large; they are peroxidase positive and stain with Sudan black B but not with PAS. The lymphocytes contain abnormal azurophilic granules which stain with PAS but not with Sudan black B and are peroxidase negative. Similar granules have been described in monocytes and plasma cells.

The abnormality affects children of either sex who usually die from infection during childhood. The children are often partial albinos with photophobia, retinal albinism, grey or light brown hair, light skin, recurrent skin infections, and hepatosplenomegaly. Less often lymph glands may be enlarged. Neurological manifestations, hyperhydrosis, and ulceration in the mouth have also been reported. Early in the disease, apart from the specific morphological changes in the white cells, there may be only a relative lymphocytosis, but subsequently anaemia, leucopenia, and thrombocytopenia may develop, and death from sepsis or haemorrhage is common. 50% of the cases show parental consanguinity.

We present here 2 further cases.

Methods

The method for PAS reaction was modified slightly from McManus (1946). Leucocyte alkaline phosphatase

staining and scoring was by the method of Hayhoe and Quaglini (1958). Sudan black B staining was by the method of Sheehan and Storey (1947). Peroxidase reaction was estimated by the Quaglini and Flemans (1958) method. Other haematological methods were as described by Dacie and Lewis (1963).

Case Reports

Case 1. He was born on February 16, 1955, and was first seen at the age of 9, when he was referred from a dental clinic with a 6-week history of gingivitis. He had had no serious illness. He had measles, mumps, and chicken-pox at the age of 8 years, without complications. He had frequent furuncles and tended to scratch his skin, and he also had recurrent mouth ulcers. He did not sweat excessively and he tanned easily in the sun without burning. For the previous 6 weeks he had noticed that his gums bled easily on brushing his teeth.

Both his parents, who were unrelated, were English, and their families had lived in London for several generations, and neither of them knew of any other racial origin.

On examination he was a well-built boy, height 133 cm., weight 30.9 kg. He had dark brown hair with a greyish sheen and brown eyes. There were several bruises over his limbs, but no purpura. On retinoscopy there was a relative absence of retinal pigment. He showed no photophobia or nystagmus. His teeth were in good condition and his gums, though not hypertrophied, were friable and inflamed. There were enlarged lymph nodes in the left anterior cervical group, the largest being 2 cm. in diameter, and these were firm, discrete, and non-tender. No other nodes were significantly enlarged. His spleen was enlarged and palpable 2 cm. below the left costal margin on inspiration, felt firm, and was not tender. There was no hepatomegaly.

Investigations. The results of the haematological and biochemical investigations are shown in Tables I and II. The Paul Bunnell, Widal, and toxoplasma dye tests were negative. Radiographs of the chest and long bones were normal. Cervical lymph node biopsy showed reactive hyperplasia of a non-specific inflammatory type, and no abnormal granules were seen in the cells. The bone-marrow obtained by sternal puncture showed normal

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TABLE I
Haematological Investigations

Investigation	Case 1	Case 2
Haemoglobin (g./100 ml.)	11.8	11.8
PCV (%)	36	35
MCHC (%)	33	32
Reticulocyte count (%)	1	1
WBC count per c.mm.	6400	5000
Neutrophil polymorphs per c.mm.	1600	800
Eosinophil polymorphs per c.mm.	64	300
Lymphocytes per c.mm.	4480	3700
Monocytes per c.mm.	128	200
Metamyelocytes per c.mm.	128	
Platelet count per c.mm.	350000	320000
ESR (Westergren)	30 mm. 1st hour	9 mm. 1st hour
Bleeding time (Duke)	3 min.	4 min.
Clotting time (Dale and Laidlaw)	2 min. 10 sec.	2 min. 40 sec.
One-stage prothrombin time (using Simplastin: Warner)	12 sec. (control 12.5 sec.)	12.5 sec. (control 12.5 sec.)
Thromboplastin screening test	8 sec. in 3 min. (control 9 sec. in 3 min.)	8.5 sec. in 3 min. (control 8.5 sec. in 3 min.)
Fibrinogen titre	Up to 1 : 512	Up to 1 : 256

cellularity with changes in the white blood cells, as described below.

The gingivitis improved without specific treatment.

TABLE II
Biochemical Investigations

Investigation	Case 1	Case 2
Plasma urea (mg./100 ml.)	30	22
Plasma sodium (mEq/l.)	135	137
Plasma potassium (mEq/l.)	3.8	4.2
Plasma chloride (mEq/l.)	104	101
Plasma bicarbonate (mEq/l.)	24	21.5
Random blood glucose (mg./100 ml.)	70	Not done
Thymol turbidity	1 unit	1 unit
Zinc turbidity	2 units	3 units
Serum bilirubin (mg./100 ml.)	< 1	< 1
Serum alkaline phosphatase (KA units)	12	20
Serum protein (mg./100 ml.)	8.2	8.2
Serum albumin (mg./100 ml.)	4.4	5.1
Serum globulin (mg./100 ml.)	3.8	3.1
Protein electrophoresis	Normal pattern	Normal pattern
Protein immunophoresis	Normal pattern	Normal pattern
Serum iron (µg./100 ml.)	59	Not done
Serum iron-binding capacity (µg./100 ml.)	400	Not done
Fasting total serum lipids (mg./100 ml.)	680	800
Fasting total cholesterol (mg./100 ml.)	190	250
Fasting free cholesterol (mg./100 ml.)	75	35
Fasting glycerides (mg./100 ml.)	80	100
Fasting phospholipids (mg./100 ml.)	250	320
Lipoprotein electrophoresis	Normal pattern	Normal pattern
Urine cells	Nil	Nil
Urine protein	Nil	Nil
Urine reducing substances	Nil	Nil
Urine acetone	Nil	Nil

Note: The serum lipid extraction was performed by the method of Bloor (1925) and the eluted lipid fractions separated by thin-layer chromatography, and estimated gravimetrically.

Investigations two months later showed that the haemoglobin was 12.3 g./100 ml., the white cell count 6500/c.mm., with 1300 neutrophils, 260 eosinophils, 4420 lymphocytes and 520 monocytes/c.mm. The ESR (Westergren) had fallen to 9 mm./1 hr.

Case 2. The sister of Case 1 was born on April 4, 1959, and was first seen at the age of 5 years, when peripheral blood films from the family were examined, and she was found to have the typical white cell changes of the Chediak-Higashi anomaly. Apart from a similar tendency to frequent furuncles, occasional mouth ulcers, and pruritus, her medical history was normal. On examination there was a close physical resemblance to her brother; her height was 114 cm. and her weight was 19 kg. Like her brother, she had brown hair with a greyish sheen and a relative absence of retinal pigment, but no photophobia or nystagmus. There were no bruises or purpura. The spleen was palpable 3 cm. below the left costal margin, felt firm, and was not tender. There was no hepatomegaly.

Investigations. The results of the haematological and biochemical investigations are recorded in Tables I and II. The Paul Bunnell, blood Wassermann, and Widal tests were negative.

The bone-marrow obtained by sternal puncture showed normal cellularity with changes in the white cells as described below.

Chromosomal analyses on both children, carried out on peripheral blood cultures by Professor P. E. Polani, were normal.

Haematological Findings

Case 1.

Leucocytes—neutrophils (Fig. 1). The cells were of normal size and their nuclei appeared normal. When stained with Leishman's stain the cytoplasm contained an average of 10 large grey-brown granules of irregular shape, ranging from 0.5–3 µ in diameter and staining with varied intensity. Smaller granules predominated, but the larger granules stained more deeply. There was no correlation between the number of these granules and the number of lobes in the nucleus. Smaller pale pink granules were also present. The large granules were not seen in the myeloblasts and promyelocytes, which contained pale pink, round bodies 0.5–2 µ in diameter. These appeared to be precursors of the large granules in the mature neutrophils, as intermediate changes were found in the myelocytes and metamyelocytes. The smaller paler bodies in the primitive cells appeared to coalesce as the cells matured, forming the large dark granules seen in the more mature cells. The neutrophils stained strongly with PAS, but there were areas of very weak or absent staining which appeared to correspond with the abnormal granules seen in Leishman preparations. Neutrophil alkaline phosphatase score was 23, and in some of the neutrophils there were clear unstained areas after staining for alkaline phosphatase, which again appeared to correspond with the abnormal granules. Sudan black B stained the abnormal granules black or

FIG. 1.—Peripheral blood. Polymorphonuclear neutrophil cell with abnormal granulations. (Leishman stain. $\times 2000$.)

dark green with a black rim; with this stain the granules were more easily seen than in Leishman preparations, as smaller granules were distinguishable and each cell contained about 20 granules. The abnormal granules were peroxidase positive.

Eosinophils (Fig. 2). The eosinophil granules were large ($1-3\mu$ in diameter) and round or ovoid in shape. When stained with Leishman's stain most of the granules

were normal in colour, but some cells contained a few granules which stained the same colour as the abnormal neutrophil granules. With PAS reagent most of the eosinophil granules appeared to be ringed; the centre of the granule did not stain. A few granules, however, stained completely. With Sudan black B eosinophil granules stained less deeply than those in the neutrophils, appearing pale green with a thin, darker staining rim to each granule. The granules were peroxidase positive.

FIG. 2.—Peripheral blood. Eosinophil with large granules. (Leishman stain. $\times 1960$.)

FIG. 3.—Peripheral blood. Lymphocyte with inclusion. (Leishman stain. $\times 1860$.)

Lymphocytes (Fig. 3). The cytoplasm contained one or occasionally two pink granules $1-2\ \mu$ in diameter when stained with Leishman's stain. These were found in 30% of the peripheral lymphocytes. They were weakly positive with PAS but negative with Sudan black B and peroxidase stains.

Monocytes. The cells showed normal granules and a few small vacuoles, but no large inclusions of the type described by Undritz (1958).

Plasma cells. When stained with Leishman's stain a few of these cells contained one or two pink granules, $0.5\ \mu$ in diameter, surrounded by a halo.

Megakaryocytes, platelets, and cells of the erythropoietic series appeared normal.

Case 2. The cells in the blood and bone-marrow showed the same characteristics and abnormalities as those described for Case 1. Only 20% of the peripheral lymphocytes contained pink granules.

Findings in the Family

The pedigree of the affected family is shown in Fig. 4. Peripheral blood was obtained from both the paternal grandparents, the maternal grandfather, the father, the mother, the 4 other children of the sibship, and the 2 children born of the mother's first marriage. All these subjects were clinically normal. In 1.5% of the lymphocytes of the maternal grandfather and the mother granules similar to, but smaller and more numerous than, those found in the lymphocytes of the affected children were seen (Fig. 5). The mother was one of twins: her twin brother died aged 4 months. She had an elder

sister who died aged 2 years, and younger twin brothers, one of whom died aged 2 hours and the other was still-born. No clinical details of these children are available. The mother had 2 abortions at 3 months' gestation during her first marriage.

Discussion

Kritzler, Turner, Lindenbaum, Magidson, Williams, Preisig, and Phillips (1964) were able to quote reports of 32 cases of the Chediak-Higashi anomaly from widely scattered areas of the world.

FIG. 4.—Pedigree of Cases 1 and 2.

FIG. 5.—Peripheral blood of maternal grandfather. Lymphocyte with inclusions. (Leishman stain. $\times 1860$.)

Since then, 2 further cases have been reported from Brazil (Cat, Marinoni, Giraldi, de Almeida, Neto, Braga, and da Silva, 1965), and one, the first from Great Britain, by Fletcher and Garvie (1965).^{*} All these cases were diagnosed in infancy or childhood and the majority died before the age of 7 years. Some of the children were ill from early childhood, but others developed normally for several years before entering an accelerated phase of the disease, with fever, hepatosplenomegaly, and pancytopenia, leading to death within a few months from haemorrhage and infection. Our 2 cases are alive and well at the ages of 6 and 10, respectively. A few children have remained healthy well into the second decade. The oldest reported case was 18 (Kritzler *et al.*, 1964).

The familial incidence and high rate of parental consanguinity in reported cases strongly suggest inheritance of an autosomal recessive gene. Slight changes have been reported in occasional lymphocytes of the parents and otherwise unaffected sibs (Béguet César, 1943; Efrati and Jonas, 1958; Saraiva, Azevedo, Correa, Carvalho, and Prospero, 1959; Kritzler *et al.*, 1964). It has been suggested that the fully developed case is homozygous and the heterozygote may sometimes be identified by demonstrating granules in a small percentage of the lymphocytes in the peripheral blood (Kritzler *et al.*,

1964). Only the mother and maternal grandfather of our 2 cases have granules in a few of their lymphocytes, and these changes have not been found in all the other parents of affected children examined or in as many of the sibs as would be expected if all heterozygotes could be identified in this way. It is, however, a familiar situation that when an ordinarily recessive gene shows lesser manifestations in the heterozygote these changes occur in only a proportion of the heterozygotes.

The anomaly has been reported in animals: in Aleutian mink and in albino cattle. The affected animals show the typical white cell changes, partial retinal albinism, photophobia, and susceptibility to infection. None of the albino cattle lived longer than four years. The anomaly is inherited in these animals as an autosomal recessive characteristic (Padgett, Leader, Gorham, and O'Mary, 1964).

Electron microscopy (Bernard, Bessis, Seligmann, Chassigneux, and Chome, 1960) demonstrates that the granules in the immature polymorphonuclear cells are normal. The abnormal granules appear as the cells mature and seem to develop as a result of fusion of the normal granules. The granules in the lymphocytes and monocytes, however, do not resemble any granules previously seen in these cell types. Cytochemical studies (Mauri and Silingardi, 1964) confirm that the chemical composition of the abnormal polymorphonuclear granules is qualitatively similar to normal granules, but differs quantitatively, and that the granules seen in the lymphocytes

^{*} Fletcher and Garvie (1965) reported on serum lipid analyses in a case of the Chediak-Higashi anomaly after this paper had been written. The patient was an 11-month-old girl, the second affected in a family of 4. No mention was made of racial origin. This makes our cases the second reported in Great Britain.

are quite unlike any normally seen in these cells. The cytochemical pattern of the abnormal granules in the various cell types is different. Necropsy findings by Kritzler *et al.* (1964) demonstrated abnormal inclusions in histiocytes, neurones, and renal tubular cells in 5 cases of the Chediak-Higashi anomaly examined by them, which suggests the possibility of a widespread storage disease.

Abnormalities of serum lipids have been reported in three cases of the anomaly. Kritzler *et al.* (1964) reported increased fasting serum triglyceride levels, relatively decreased concentrations of lysolecithin and α_1 -lipoproteins, and an increase in the ratio of free to total cholesterol in the propositus and in 2 members of her immediate family; the ratio of fasting serum lecithin to sphingomyelin was increased in the propositus and all members of her immediate family. They failed to demonstrate these changes in one other unrelated case studied. Cat *et al.* (1965) described hyperlipaemia in association with the anomaly in 2 brothers, one of whom had a high blood neutral fat but a normal cholesterol level. Lipid analysis in our first case gave results well within normal limits, but in the second case the concentrations of the fasting total lipids, cholesterol, and phospholipids were at the upper limit of normal and further studies are being carried out.

Another common feature of the affected children is their increased susceptibility to infection, from which most of them die. Investigations have shown a normal antibody response following immunization and normal γ -globulin levels. Studies of leucocyte function demonstrate normal migration of cells to a site of inflammation and normal *in vitro* phagocytosis of bacteria when incubated with cultures of *Salmonella typhosa* and *Staphylococcus pyogenes* (Page, Berendes, Warner, and Good, 1962; Saraiva *et al.*, 1959). Leucopenia is a frequent manifestation of the anomaly and neutropenia is present in both cases reported here. This may account for the increased susceptibility to infections and for the recurrent oral ulceration, as in cyclic neutropenia (Gorlin and Chaudhry, 1960). We have not been able to demonstrate any cyclic variation in the neutrophil counts in our cases, which seem persistently low. A reduced neutrophil life-span due to an inherent defect in the cell (perhaps associated with the enlarged spleen) or deficient production by the bone-marrow could account for the neutropenia.

Summary

Two patients with the Chediak-Higashi anomaly are described, a brother and sister aged 10 and 6. The salient features in both cases were brown hair with a greyish sheen, partial retinal albinism,

splenomegaly, and a tendency to skin infections and oral ulceration. Both children showed an absolute neutropenia and relative lymphocytosis, and their white blood cells showed the changes typical of the Chediak-Higashi anomaly. The two children have remained well during the one year they have been observed.

Abnormal granules were present in the lymphocytes of the mother and maternal grandfather.

The prognosis and inheritance, the cytochemical changes, and their significance are discussed.

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