

CYTOLOGICAL, CYTOCHEMICAL AND IMMUNOLOGICAL  
FINDINGS FROM TWO CHILDREN WITH CHEDIAK-HIGASHI  
SYNDROME\*

Adele CATERINO-de-ARAÚJO\*\*  
Elizabeth de los SANTOS-FORTUNA\*\*  
Magda Maria de Sales CARNEIRO-SAMPAIO\*\*\*

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**ABSTRACT:** This article describes studies of two unrelated patients, aged near 2 years, with leukocyte granulation abnormalities similar to those presented in the Chediak-Higashi Syndrome (CHS). Both patients showed dermatologic manifestations characterized by hypopigmentation of the hair and skin. The cytological and cytochemical study of the peripheral blood leukocytes demonstrated giant abnormal granules with lisosomal content presented in neutrophils, eosinophils and monocytes. The mononuclear cells displayed one large azurophil granule. The cytochemical functional study of neutrophils showed normal nitroblue-tetrazolium reduction test. The immunological status of the patients were made and in patient 1 was detected a low number of T helper lymphocytes and a maintenance of lymphocytes in continuous cell culture for five weeks without addition of external growth factor. These findings with clinical manifestations permitted to establish the CHS, in these cases.

**DESCRIPTORS:** Chediak-Higashi Syndrome (CHS); peripheral blood leukocytes; abnormal granules; cytological, cytochemical and immunological study; lymphocyte subsets.

INTRODUCTION

Chediak-Higashi Syndrome (CHS) is an inherited autosomal recessive generalized cellular disorder characterized by partial albinism (depigmentation of eye, hair and skin), frequent pyogenic infections and abnormal large granules in leukocytes and other granule-containing cells. CHS patients frequently exhibit neutropenia, relative lymphocytosis, thrombocytopenia, nystagmus, peripheral neuropathy, fever of unknown origin and impairment of natural killer cells function. The disease evolves to an accelerated phase with pancytopenia and a diffuse mononuclear infiltrate<sup>3</sup>. This rapidly proliferative phase

usually leads to death from infection or hemorrhage, and may be associated with a T-cell lymphoma<sup>2</sup>.

The identification of abnormally large cytoplasmic inclusions in circulating white blood cells and bone marrow is a very important morphologic marker for CHS<sup>4,11</sup>. These inclusions are shown as multiple irregular grey to dark blue granules in neutrophils and one large azurophil granule in lymphocytes and monocytes, when stained with Wright's stain. In neutrophils, cytochemical studies have shown that these granules were positive for peroxidase, acid phosphatase and esterase.

\* Realizado na Seção de Imunologia do Instituto Adolfo Lutz, São Paulo, SP.

\*\* Da Seção de Imunologia do Instituto Adolfo Lutz, São Paulo, SP.

\*\*\* Do Departamento de Imunologia Clínica, Instituto da Criança "Prof. Pedro de Alcântara", Hospital das Clínicas, Universidade de São Paulo, SP.

In this report, we describe two unrelated patients with clinical, morphologic and cytochemical features of leukocytes similar to those from patients with CHS. One of them showed also lymphocytosis.

## CASES AND METHODS

### *Patient 1*

Patient 1 is a 2.1-yr-old mulatto male who was a 7 pound, 8-ounce, 40-week-gestation product of a 31-yr-old woman. Neonatal cyanosis during the period of 24 hours and nasal obstruction were noted. At age of 2 months the patient presented bronchopneumonia and at 5 months he was found to have hypopigmented skin and hair.

The family medical history reported a brother and a cousin who died after presenting the same cutaneous picture. He has two normal brothers and two normal sisters. The parents are cousins.

The patient has no history of other serious infections. However, he did experience repeated bouts of furunculosis.

The child was referred to the Instituto da Criança "Prof. Pedro de Alcântara", Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo and the Chediak-Higashi syndrome was diagnosed by the examination of the blood and bone marrow samples that revealed large, abnormal granules in most granulocytes and in many lymphocytes and monocytes.

At that time, the patient was found to have enlarged spleen and liver, moderate anaemia and otitis media. He was treated with ferrous sulphate, antibiotics and ascorbic acid (1g/day). After then, he showed a transient period of lymphocytosis.

### *Patient 2*

Patient 2 is a 2-yr-old mulatto girl who has no family history since her parents were anophthalmic. She is adoptive daughter of a rich family.

The patient was referred to the "Instituto da Criança Prof. Pedro de Alcântara, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo" due to dermatologic problems. On admission the child presented partial albinism with depigmentation of eye, hair and skin, photophobia, nystagmus, intermittent febrile episodes and pyogenic infections. The presence of abnormal large granules in the cytoplasm of peripheral leukocytes and bone marrow finally led to the diagnosis of CHS.

Nowadays, she is in a good health, with no signs of the accelerated phase of the disease.

### *Laboratorial Methods*

Peripheral blood and bone marrow smears were processed by Leishman stain. The peripheral blood leukocytes were stained for acid phosphatase, alkaline phosphatase, peroxidase, mucopolysaccharides (PAS), neutral fat (Sudan Black), nonspecific esterase using alpha-naphthyl butyrate as substrate<sup>19</sup>. The nitroblue-tetrazolium (NBT) reduction test, stimulated or not with lipopolysaccharide, was also performed<sup>10</sup>. All reagents used were from SIGMA Co., U. S. A.

Mononuclear cells from peripheral blood were isolated by centrifugation on a ficoll-hypaque gradient. The number of T and B was determined by the rosette method using sheep red blood cells and zymosan-complement complex<sup>1</sup>.

Determinations of the subset lymphocytes bearing the CD4 or CD8 marker were made by staining cells with a fluorescein conjugated monoclonal antibody (Leu 3a and Leu 2a, Becton-Dickinson, U. S. A.), using a direct immunofluorescence assay<sup>1</sup>.

The lymphocytes of the patient 1 were stimulated with phytohemagglutinin mitogen and also maintained in suspension culture for five weeks, without addition of external growth factors<sup>5</sup>. The medium was changed weekly by a fresh one through centrifugation of the cells in culture. The viability was determined by trypan blue exclusion technique.

## RESULTS

### *Cytological study*

The bone marrow smears showed abnormal granules in megakaryocytes, promyelocytes, myelocytes and leukocytes in both cases.

Light microscopic and cytochemical studies from peripheral blood leukocytes showed the characteristic lysosomal granules, as previously described in CHS.

The neutrophil and eosinophil cells stained by Leishman and Rosenfeld stains displayed variable number, size, shape and color of the granules in both patients (Figure 1A, B).

Patient 1 presented 90% of neutrophils with abnormal granules, ranging from 5 to 10 per cell and with variable electron dense content. Approximately 30% of the lymphocytes had one

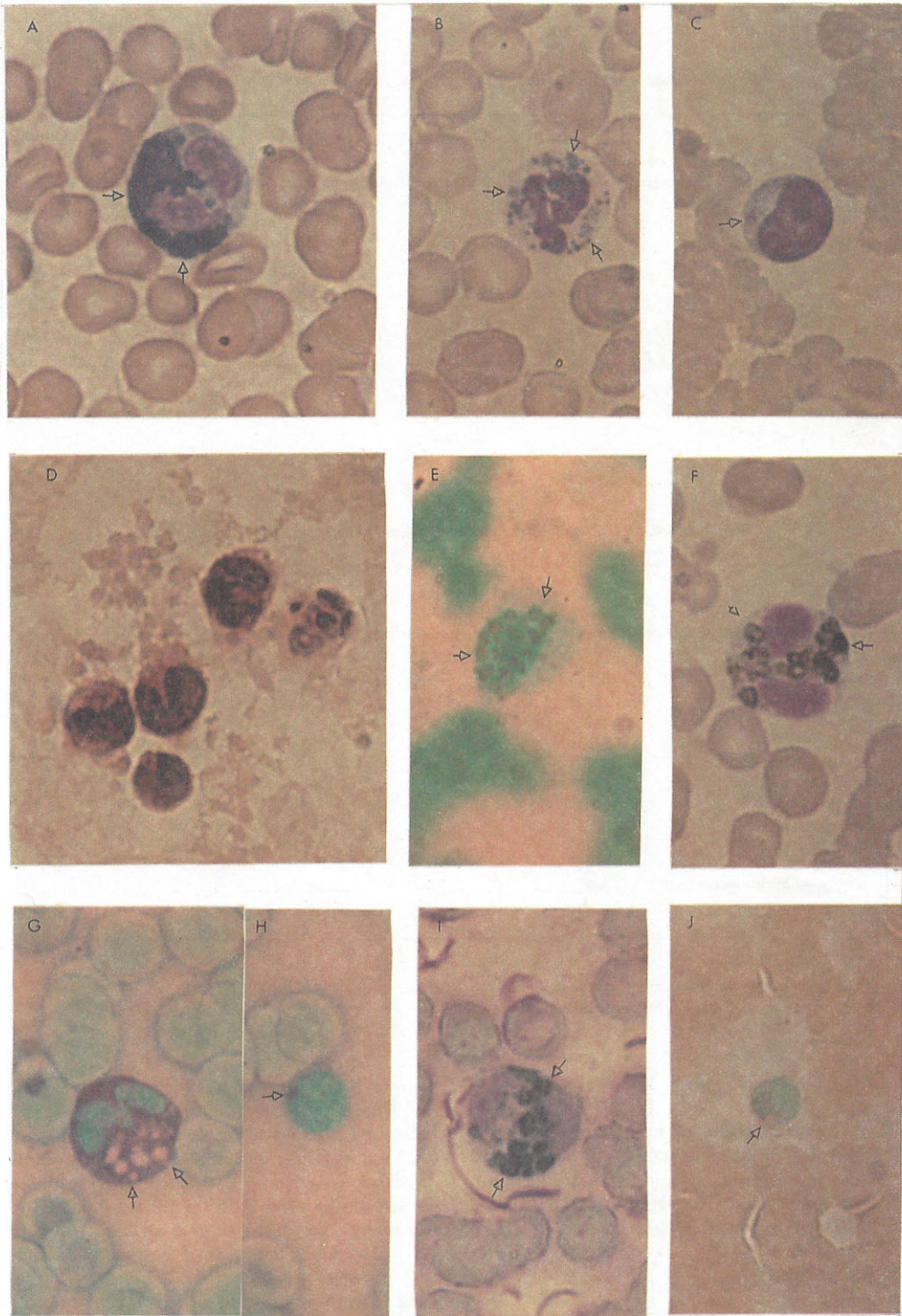


FIGURE 1 — Morphology and cytochemistry of Chediak-Higashi's abnormal granules indicated by arrows: A- eosinophilic granulocyte, B- neutrophilic granulocyte, C- lymphocyte (Leishman stain x 1000), D- positive acid phosphatase in leukocytes, E- positive alkaline phosphatase in neutrophilic granulocyte, F- positive peroxidase reaction in neutrophilic granulocyte, G- negative PAS in neutrophilic granulocyte, H- positive PAS in lymphocyte, I- positive Sudan-black reaction in neutrophilic granulocyte, J- positive nonspecific esterase in lymphocyte.

TABLE 1

*FCytochemical characteristics of leukocytes granules from the two patients.*

	acid phosphatase	alkalin phosphatase	peroxidase	P.A.S	Sudan Black	nonspecific esterase
neutrophil	pos/neg	pos	pos	neg	pos	pos
eosinophil	pos/neg	pos	pos	neg	pos	pos
monocyte	pos/neg	neg	pos	pos	pos	pos
lymphocyte	pos/neg	neg	neg	pos	neg	pos

pos = positive.  
neg = negative.

TABLE 2

*White blood cell count and lymphocytes subsets from the two patients.*

	Patient 1		Patient 2	
leukocytes x 10 <sup>6</sup> /l	6.700	50,700	8,400	10,800
neutrophils %	32,0	5,0	29,0	36,0
eosinophils %	0,0	3,0	0,0	2,0
monocytes %	10,0	4,0	3,0	2,0
lymphocytes %	58,0	88,0	68,0	60,0
lymphocytes x 10 <sup>6</sup> /l	3,886	50,116	5,712	6,480
T lymphocytes x 10 <sup>6</sup> /l	N.D.*	25,545	N.D.	2,981
T lymphocytes %	N.D.	45,0	N.D.	46,0
B lymphocytes x 10 <sup>6</sup> /l	N.D.	4,008	N.D.	842
B lymphocytes %	N.D.	8,0	N.D.	13,0
T helper cells % **	N.D.	22,0	N.D.	N.D.
T suppressor cells % ***	N.D.	17,0	N.D.	N.D.
T helper / T suppressor	N.D.	1,2	N.D.	N.D.

\* N.D. = not done.

\*\* T helper cells = CD4+ lymphocytes.

\*\*\* T suppressor cells = CD8+ lymphocytes.

purple stained body (Figure 1C).

Patient 2 showed 81% of neutrophils with 16 to 25 abnormal granules and 100% of eosinophils with a median of 40 inclusion bodies per cell. Forty percent of lymphocytes contained one large granule were around 40.

#### Cytochemical Study

The results of the cytochemical reactivity of the granules in the leukocytes are summarized in Table 1.

The reactivity of the neutrophil, eosinophil, lymphocyte and monocyte granules for acid phosphatase was positive (Figure 1D) and nega-

tive. Alkalin phosphatase reaction was negative in lymphocytes and monocytes and positive in neutrophils (Figure 1E). Except in lymphocytes, all abnormal granules are strongly peroxidase-positive (Figure 1F). Abnormal granulations in neutrophils were PAS negative, while the surrounding cytoplasm stained strongly pink (Figure 1G). The giant granules of lymphocytes and monocytes gave positive PAS reaction (Figure 1H). There were some variation in the intensity of the reactivity of the granules with Sudan-Black being strongly positive in neutrophils, eosinophils and monocytes (Figure 1I), and negative in the lymphocytes. Esterase activity of the granule was shown as a large, reddish brown aggregate (Figure 1J) resulting from a strong enzymatic activity

and also as a weak diffuse staining in the cytoplasm of neutrophils and monocytes.

The NBT histochemical stimulated test, using lipopolysaccharide from *E.coli* was positive in 58% of neutrophils from patient 1 and 88% in neutrophils from patient 2.

#### *Immunological evaluation*

The results of the quantitative analysis of the blood cells are shown in Table 2.

The CHS patients showed a low neutrophils count.

The patient 1 exhibit leukocytosis and lymphocytosis during a period of the disease, when his T and B lymphocytes were diminished in percentage and increased in total number. The patient 2 displayed similar results of T and B lymphocytes in percentage but in absolute number there were normal. The lymphocytes subsets were found altered with a low number of T helper cells in patient 1 when compared with a control group.

The patient 1 was studied three times. In the second one he showed an episode of lymphoproliferation, returning to normal after two months.

Normal values obtained from twenty healthy children aged from four months to fourteen years old without clinical and immunological disorder were: lymphocytes 3,165 x 10<sup>6</sup>/l, T lymphocytes 60%, B lymphocytes 15%, T helper cells 39.5%, T suppressor cells 25%, T helper/T suppressor ratio= 1.7.

The mononuclear cells obtained from patient 1 during the lymphoproliferative phase showed normal response to the PHA and remained alive in continuous culture for five weeks, with a viability ranging from 96 to 60%. The cells death in the culture was due to fungal contamination.

## DISCUSSION

In this study two cases of CHS are presented. Both are mulatto children from the North East region of Brazil where the consanguineous marriages are frequent, as referred in familial history of patient 1.

The clinical manifestations frequently reported in CHS were noted in these patients. They showed skin hypopigmentation and silvery tint color of the hair<sup>3,9</sup>. Patient 2 mentioned a susceptibility to sunlight and had also photophobia and rotatory nystagmus. Both patients referred repeated febrile

episodes with recurrent infectious diseases.

An interview with the mother of patient 1 revealed the death of another son and nephew with the same cutaneous picture.

The cutaneous hypopigmentation seen in these patients are specially related to giant melanosomes present in melanocytes<sup>3</sup>.

The genetic involvement in this disorder is determined by one recessive gene that is lethal in homozygous state<sup>17</sup>. The karyotype has been found normal in number of chromosome but with prevalent breakages in chromatide<sup>15</sup>. The consanguineous marriages are frequent in these cases<sup>6</sup>.

In both cases of this paper, the CHS was diagnosed by laboratorial pathognomonic finding of abnormal granules present in peripheral blood and bone marrow leukocytes, similar to those previously described in morphological and cytochemical tests<sup>4,11,15</sup>.

Variations in number, size, shape and color of the granules were displayed in polymorphonuclear leukocytes. The lysosomal nature of the granules was demonstrated by their acid phosphatase, peroxidase and esterase content. The amount of reaction product deposited in the abnormal granules and its distribution were extremely variable, even in the same cell, and this is probably associated with the presence of primary and secondary lysosomes. An explanation for the discrepancy in cell levels of lysosomal enzymes in CHS was previously demonstrated and revealed that most of the giant granules were lysosomes, loaded with substances derived from specific granules and cytoplasmic materials<sup>18</sup>.

Although in this study the majority of leukocytes showed large abnormal granules, the respiratory burst activity was normal, as demonstrated by a normal stimulated NBT test. Similar results were described in two patients with leukocytes granulation abnormalities associated with neurologic impairment, without other signs of CHS<sup>8</sup>.

Abnormalities of cyclic nucleotide metabolism, disorders of microtubule assembly, impaired neutrophil and monocyte chemotaxis, and delayed phagolysosomal fusion are related to the increased susceptibility of these patients to infection and justify the classification of this entity as a "phagocyte disorder"<sup>13,14,18</sup>.

In order to correct the microtubule defect and membrane fluidity in both patients, megadoses of ascorbic acid was used<sup>3,9</sup>. A transient reduction in the number of abnormal granules in leukocytes was observed in patient 1. On the other hand, after six months follow-up he developed a



marked leukocytosis and lymphocytosis. Despite of the increased number of lymphocytes, CD4+ lymphocytes were diminished in percentage. In addition to this, the mononuclear cells obtained from patient 1 at this time were capable of remaining alive for five weeks in continuous cell culture, without additional conditioned medium.

Several reports suggest that lymphoproliferative phase of the disease is a reaction to a viral infection attributed to Epstein-Barr virus, Cytomegalovirus, Herpes simplex virus or to Varicella-zoster virus<sup>3,5,7,12,16</sup>; however, direct evidence for this generally lacking. The basis for the marked cellular proliferation is not understood. In the present study it was not possible to demonstrate virus-like particle in peripheral blood cells nor to perform serological tests.

Others observations do not exclude the possibility of a reactive process which coexist with a neoplastic one. So, lymphomas Hodgkin's and the T-cell type were described<sup>2</sup>. Some patients experience repeated lymphoproliferative phases interrupted by periods of remission. Our patient 1 presented fever, jaundice, hepatosplenomegaly, lymphadenopathy and pancytopenia and at a peripheral level he presented a mixed population of lymphoid cells and not a homogeneous population of highly atypical cells. This is consis-

tent with a benign reactive process.

Continuous cell lines from peripheral blood lymphocytes were established from one male patient with CHS (homozygous line) and from his father (heterozygous line), although no fungal, bacterial and viral material could be recovered<sup>5</sup>. It's not known, whether viral agents are present "in vivo" in the cells obtained for culture or released from a latent stage upon being subjected to "in vitro" conditions.

Little is known about patient 2 since her parents are anonymous. Nevertheless, both patients have been followed, and a bone marrow transplant is being evaluated as an approach to correct a series of abnormalities that involves hematopoietic progenitor cells.

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RIAL6/689

CATERINO-de-ARAÚJO, A.; SANTOS-FORTUNA, E. de los & CARNEIRO-SAMPAIO, M. M. de S. — Estudo citológico, citoquímico e imunológico de duas crianças com síndrome de Chediak-Higashi. *Rev. Inst. Adolfo Lutz*, 50(1/2): 261-267, 1990.

RESUMO: Este trabalho teve por objetivo relatar os resultados obtidos do estudo clínico e laboratorial de duas crianças não aparentadas com idade aproximada de dois anos que apresentavam leucócitos com granulações anormais no sangue periférico semelhantes às observadas na Síndrome de Chediak-Higashi (SCH). Em ambos os casos havia manifestação dermatológica caracterizada por hipopigmentação da pele e cabelos. O estudo citológico e citoquímico das células do sangue dessas crianças mostrou grande número de grânulos gigantes que continham enzimas lisossomais em neutrófilos e eosinófilos. As células mononucleares apresentaram um único grânulo gigante azurófilo. O estudo citoquímico funcional dos neutrófilos mostrou capacidade normal de redução do corante "nitrobluc-tetrazolium" (NBT). O estudo fenotípico das populações linfocitárias nesses casos revelou diminuição no número de linfócitos T auxiliares no paciente 1, durante uma fase de linfoproliferação. Esses linfócitos permaneceram viáveis por cinco semanas em cultura sem adição externa de fatores de crescimento celular. Os resultados obtidos permitiram o diagnóstico de SCH nessas crianças.

DESCRITORES: Síndrome de Chediak-Higashi (SCH); leucócitos do sangue periférico; granulações anormais; estudo citológico, citoquímico e imunológico; subpopulações de linfócitos.

REFERENCES

1. ARAUJO, A. C. — Avaliação "in vitro" da resposta imune mediada por células na Síndrome de Imunodeficiência Adquirida—AIDS. São Paulo, 1985. [Masters Thesis. Escola Paulista de Medicina, São Paulo] p.136.
2. ARGYLE, J. B.; KJELDSBERG, C. R.; MARTY, J.; SHIGEOKA, A. O. & HILL, H. R. — T-Cell lymphoma and the Chediak-Higashi syndrome. *Blood*, 60:672-676, 1982.
3. BARAK, Y. & NIR, E. — Chediak-Higashi syndrome. *Amer. J. Pediatr. Hematol. Oncol.*, 9: 42-55, 1987.
4. BESSIS, M.; BERNARD, J. & SELIGMANN, M. — Étude cytologique d'un cas de maladie de Chediak. *Nouv. Rev. fr. Hem.*, 1: 422-440, 1961.
5. BLUME, R.S.; GLADE, P.R.; GRALNICK, H.R.; CHESSIN, L.N.; HAASE, A.T. & WOLFF, S.M. — Chediak-Higashi syndrome: continuous suspension cultures derived from peripheral blood. *Blood*, 33: 821-832, 1969.
6. BLUME, R.S. & WOLFF, S.M. — The Chediak-Higashi syndrome: studies in four patients and a review of the literature. *Medicine*, 51: 247-280, 1972.
7. DOUGLAS, S.D.; BLUME, R.S.; GLADE, P.R.; CHESSIN, L.N. & WOLFF, S.M. — Fine structure of continuous long term lymphoid cell cultures from a Chediak-Higashi patient and heterozygote. *Lab. Invest.*, 21: 225-229, 1969.
8. GALE, P.F.; PARKIN, J.L.; QUIE, P.G.; PETTIT, R.E.; NELSON, R.P. & BRUNNING, R.D. — Leukocyte granulation abnormality associated with normal neutrophil function and neurologic impairment. *Amer. J. clin. Path.*, 86: 33-49, 1986.
9. GIACOIA, G.P.; CHOATE, B.P.; GELLIS, S.S. & FEINGOLD, M. — Chediak-Steinbrinck-Higashi syndrome. *Amer. J. Dis. Child.*, 135: 949-950, 1981.
10. INSTITUTO ADOLFO LUTZ — SÃO PAULO. — *Normas Analíticas do Instituto Adolfo Lutz*. 2ª ed., São Paulo, Tip. Fonseca, 1976.
11. MAURI, C. & SILINGARDI, V. — A cytological and cytochemical study of Chediak's leukocytic anomaly. *Acta haemat.*, 32: 114-126, 1964.
12. MERINO, F.; PAUZA, M. & PURTILO, D.T. — Anti-Epstein-Barr virus memory T-cell response in Chediak-Higashi syndrome patients. *Immunol. Letters*, 12: 51-54, 1986.
13. MILLER, A.L.; STEIN, R.; SUNDSMO, M. & YEH, R.Y. — Characterization of lysosomal enzymes from Chediak-Higashi syndrome cultured fibroblasts. *Biochem. J.*, 238: 589-595, 1986.
14. ROTROSEN, D. & GALLIN, J.I. — Disorders of phagocyte function. *Ann. Rev. Immunol.*, 5: 127-150, 1987.
15. ROZENSZAJN, L.A. & RADNAY, J. — The lysosomal nature of the anomalous granules and chromosome aberrations in cultures of peripheral blood in Chediak-Higashi syndrome. *Brit. J. Haematol.*, 18: 683-689, 1970.
16. RUBIN, C.M.; BURKE, B.A.; McKENNA, R.W.; McCLAIN, K.L.; WHITE, J.G.; NESBIT, M.E. & FILIPOVICH, A.H. — The accelerated phase of Chediak-Higashi syndrome. An expression of the virus-associated hemophagocytic syndrome. *Cancer*, 56: 524-530, 1985.
17. SADAN, N.; YAFFE, D.; ROZENSZAJN, L.; ADAI, H.; SOROKER, B. & EFRATI, P. — Cytochemical and genetic studies in four cases of Chediak-Higashi-Steinbrinck syndrome. *Acta haematol.*, 34: 20-29, 1965.
18. WHITE, J.G. & CLAWSON, C.C. — Chediak-Higashi syndrome: variable cytochemical reactivity of giant inclusions in polymorphonuclear leukocytes. *Ultrastructural Pathol.*, 1: 223-226, 1980.
19. YAM, L.T.; LI, C.Y. & CROSBY, W.H. — Cytochemical identification of monocytes and granulocytes. *Amer. J. clin. Path.*, 55: 283-290, 1970.

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