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Chediak–Higashi Syndrome: Immunological Responses to Epstein–Barr Virus Studies in Gene Heterozygotes

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Immunologic studies were performed in five fathers and nine mothers of patients with Chediak-Higashi Syndrome (CHS). Antibody response to Epstein-Barr virus capsid antigen was higher than in normal controls. Antibodies to diffuse component of the early antigen were not detected and serum antibodies to the restricted component of the early antigen were observed in 64% of the subjects studied. Low natural killer activity and increased proportions of OKT8 positive cells were increased. These data indicate that immunologic alterations similar to those seen in CHS patients can be observed in their asymptomatic parents.

KEY WORDS: Chediak–Higashi syndrome; Epstein–Barr virus.

INTRODUCTION

The Chediak–Higashi syndrome (CHS) (1) is a rare autosomal recessive genetic disease characterized by predisposition to pyogenic infections, partial albinism, ash-gray hair color, abnormal bactericida neutrophil function, and large cytoplasmic masses in all body granule-producing cells. Diagnosis is made by the observation of large azurophilic cytoplasmic granules in peripheral blood neutrophils and lymphocytes (2, 3). In addition to the alteration in the neutrophil digestive capacity, absent or severely reduced natural killer (NK)-cell activity, antibody-dependent cell-mediated cytotoxicity (ADCC) (4, 5), and lectin-mediated cytotoxicity (6), as well as an altered number and function of peripheral blood lymphocyte subsets (7, 8) and abnormal responses to Epstein–Barr virus (EBV) infection have been described in these patients (5).

In known heterozygous subjects for the CHS gene, in addition to the typical CHS large cytoplasmic granules in peripheral blood cells, modifications such as abnormal lysosomal enzymatic contents, intracytoplasmic zinc concentrations, and serum lipids, decreased chemotactic activity of polymorphonuclear neutrophils, and skin abnormalities have been reported (10–13). Detailed immunological studies on heterozygotes have not been reported in the literature because CHS is a rare disease. However, the recently described cluster in the Venezuelan Andes enables us to study a large group of parents, all of similar genetic origin and within the same environmental conditions.

We now report studies performed in parents, known heterozygous for the CHS gene, of patients from the recently described cluster of this disease in Venezuela. It was found that immunological abnormalities, i.e., low NK-cell activity and abnormal EBV-specific serological pattern, can be detected in the CHS gene carriers.

MATERIALS AND METHODS

Five fathers and nine mothers of Chediak–Higashi syndrome patients were available for study. All patients were from the cluster of this disease in the State of Táchira, Venezuela (14). All families were residing in communities of this state and within a geographical area of about 100 km^2. Clinical details of the CHS patients have been published elsewhere (5, 7; F. Merino, submitted for publication). Diagnosis was made by the clinical findings of partial albinism, ash-gray hair, photophobia, and

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the presence of large azurophilic, peroxidase-positive, cytoplasmic granules in neutrophils and lymphocytes and by the demonstration of an abnormal digestion of *Candida albicans* by the granulocytes.

Absolute numbers of peripheral blood white cells were calculated by differential count in Wright-stained blood smears.

Mononuclear cells were isolated from the heparinized peripheral blood by density-gradient, Ficoll–Hypaque centrifugation. Lymphocyte populations were determined as the number of rosetting cells with sheep erythrocytes or surface immunoglobulin-positive lymphocytes in an immunofluorescence assay. Lymphocyte subsets were characterized by their reactivity with monoclonal antibodies to membrane surface antigens. Monoclonal antibodies OKT3 (pan-T), OKT4 (T helper), OKT8 (T suppressor/cytotoxic), and OKIa-1 (HLA-DR) were from Ortho Diagnostics (Raritan, NJ). B1 monoclonal antibody was from Coulter (Hialeah, FL), and Leu-7 from Becton–Dickinson (Sunnyvale, CA). They were used according to the manufacturers' instructions.

The *in vitro* proliferative response to mitogen stimulation was measured by the incorporation of tritiated thymidine (^{3}H-TdR) into cultured peripheral density-separated mononuclear cells. Mononuclear cells (5×10^6) were cultured in RPMI-1640 medium supplemented with antibiotics, 15% autologous plasma, and an optimal stimulating dose of concanavalin A (Con A) or pokeweed mitogen (PWM). Cells were transferred to glass-fiber filters and the radioactivity present was determined by liquid scintillation.

Natural killer (NK)-cell radioactivity was determined in a 4-hr assay by measuring the specific chromium-51 (^{51}Cr) release of labeled K562 cells, a NK-sensitive myeloid leukemia cell line. The percentage specific ^{51}Cr release was calculated according to the formula

$$\% \text{ specific release} = \frac{\text{test release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100.$$

Lectin-mediated cytotoxicity was studied by the determination of the activity of density gradient-separated mononuclear cells against K562 target cells with the addition of 1:400 diluted phytohemagglutinin (PHA-P; Wellcome Research, England) to the effector/target mixture and according to Klein *et al.* (15). Isotope release was evaluated after a 4-hr incubation period at 37°C.

Antibodies to Epstein–Barr virus capsid antigen (VCA), the restricted (EA-R) or diffuse (EA-D) components of the early membrane antigen complex, and the EBV-associated nuclear antigen (EBNA) were determined by indirect or anticomplement immunofluorescent assay (16, 17).

Hepatitis B surface antigen, antibodies to it, the subtypes Ad and Av, and the hepatitis B core antigen were determined by solid-phase radioimmunoassays. Antibodies to human T-cell leukemia/lymphoma virus (HTLV-I) were determined by the enzyme-linked immunosorbent assay (ELISA) technique (18).

Rheumatoid factor, nuclear, microsomal, mitochondrial, smooth muscle, and parietal-cell antibodies were determined using commercial kits according to manufacturers' instructions. Antilymphocytotoxic antibodies were assayed in sera of 13 parents using the standard complement-mediated two-stage NIH-Terasaki micromethod (19). T and B lymphocytes separated in nylon-wool columns from mononuclear cells from the peripheral blood of 15 healthy donors were used as targets, with incubation periods of 90 and 120 min, respectively, at room temperature.

RESULTS

Microscopic peripheral blood examination revealed the existence of large azurophilic cytoplasmic granules in the lymphocytes of almost all parents studied. Atypically large cytoplasmic granules in the cytoplasm of granulocytes could also be observed. However, the percentage of peripheral blood cells manifesting the cytoplasmic anomalies (1–2% or less) was lower than in the CHS patients (all neutrophils and about 25% of the lymphocytes). Similar cytoplasmic azurophilic granules were not observed in peripheral blood cells from normal control subjects from the same region. In electron microscopic studies the presence of large cytoplasmic vacuoles or granules could be observed in the polymorphonuclear neutrophils of both parents. Large lysosomal accumulations were also seen in the cytoplasm of lymphocytes.

The total white blood cell count and the number of peripheral blood cells were within the normal range for the healthy population of the State of Táchira, and no leukopenia, neutropenia, or lymphopenia was observed. In the parents of CHS

Table I. Peripheral Blood Lymphocyte Populations and Subsets in Chediak–Higashi Syndrome Heterozygotes

		Mean percentage positive cells									
Subject	ND[a]	SR	SIg	T3	T4	T8	T4/T8	Mo-1	Ia-1	B1	Leu-7
Mothers											
CY	3	88	14	91	49	40	1.2	21	22	9	
ER	3	77	17	86	55	34	1.6	22	16	13	
HO	2	83	17	82	39	45	0.9	26	17	9	22
IR	1	72	13		44	28	1.6	19	19		20
MGR	3	76	9		38	33	1.2	28	14		
MS	1	73			48	50	0.9	24			
Fathers											
JRG	2	95	6	95	46	34	1.4	26	17	11	34
DV	1	52	16		35	22	1.6	34			
JUR	3	78	16	83	60	26	2.3	31	11	11	15
Controls		50–90		66–84	33–50	12–24		7–23	8–17	4–8	8–20

[a]ND, number of determinations; SR, spontaneous rosetting cells with sheep erythrocytes; SIg, surface immunoglobulin-positive lymphocytes.

patients the percentage as well as the absolute number of T and B cells in peripheral blood was found to be within the normal range. OKT8-positive lymphocytes were increased in three mothers and were in the upper-normal range in the other two mothers tested. The T4/T8 ratio was within normal limits. The numbers of OKMo-1 (monocytes)-, Ia-like-, and Leu-7 (NK cells)-positive cells were found to be normal (Table I). Normal values for the general Venezuelan population are as follows: OKT3, 57–75%; OKT4, 35–50%; OKT8, 12–24%; 4/8 ratio, 1.1–2.5; OKM1, 7–23%, and OKJaI, 8–17%. Levels of serum immunoglobulins IgG, IgA, and IgM were within normal limits (5). The observed proliferative responses of peripheral blood lymphocytes to *in vitro* stimulation with concanavalin A, a T-cell mitogen, or PWM, a B-cell mitogen, were similar to those of healthy control subjects. Similar observations were made when the proliferative responses to mixed lymphocyte culture stimulation were studied (data not shown).

The results of the study of peripheral blood lymphocyte natural killer-cell cytotoxic activity are presented in Table II as the means of several determinations. A decreased lytic activity, as evidenced by a lower percentage of specific ^{51}Cr release, was observed in four of the six mothers studied. Two fathers showed cytotoxicity values similar to those of control subjects. Lectin-mediated cytotoxicity by peripheral blood lymphocytes from both mothers and fathers was found to be normal (data not shown).

The Epstein–Barr virus serum antibody response in heterozygous subjects is presented in Table III. Antibody titers to the viral capsid antigen (VCA) were found to be elevated compared to the normal range for Venezuelan populations (20). However, two mothers, ER and HO, had significantly elevated antibody titers to VCA, as did one father, JUR. Antibodies to the diffuse component of the early antigen complex were not detected and serum antibodies to the restricted component of the early membrane antigen complex (EA-R) were observed in 64.3% of the parents. This is in contrast to a frequency of 9.6% in healthy subjects. Two mothers, ER and HO, manifested elevated serum antibody titers to the EA-R antigen. Antibody titers to the Epstein–Barr nuclear antigen (EBNA) were in the normal range.

These results indicate that unusual EBV-specific serological responses can be observed in the parents of CHS patients. The patterns of high anti-

Table II. Natural Killer-Cell Activity in Chediak–Higashi Syndrome Heterozygotes

			% specific ^{51}Cr release at effector/target ratio			
Subject	Kinship	ND[a]	50/1	25/1	12/1	6/1
MGR	Mother	4	25	15	8	6
ER	Mother	3	19	13	12	10
HO	Mother	5	32	25	17	11
PM	Mother	3	9	8	4	3
CY	Mother	2	26	15	11	9
IR	Mother	2	15	14	11	4
JBT	Father	2	37	30	23	14
JUR	Father	3	44	36	28	23
Control		13	37	31	20	14

[a]Number of determinations.

Table III. Anti-Epstein–Barr Virus Serum Antibodies in Chediak–Higashi Syndrome Heterozygotes

Subject	Kinship	ND[a]	Mean geometric titer of IgG anti-Epstein–Barr virus antibodies to VCA	EA-D	EA-R	EBNA
CY	Mother	5	485	<10	10	15
ER	Mother	10	2743	<10	320	65
GDP	Mother	1	80	<10	<10	20
HO	Mother	7	780	10	98	73
IR	Mother	2	320	<10	10	80
MGR	Mother	7	238	<10	12	7
MS	Mother	2	453	<10	20	80
PM	Mother	10	86	<10	<10	98
AN	Mother	2	226	<10	20	65
JRG	Father	2	26	<10	<10	5
DV	Father	2	40	<10	<10	7
JBT	Father	2	113	<10	<10	80
PC	Father	1	320	<20	20	<2
JUR	Father	10	1196	<10	26	46
Controls		47	77	<10	<10	15

[a]Number of determinations.

VCA and high anti-EA-R antibodies observed in two mothers were similar to the serological pattern developed by CHS patients.

Hepatitis B virus surface antigen (HBsAg) was not detected in the tested sera. Antibodies to the subtypes Ad and Ay were observed in one of the six mothers studied (PM) at high titers. Serum anticore antibodies were observed in one of the three fathers tested and in one mother (PM). The frequency of HBsAg and anti-HbsAg in 35 sera from control subjects from the same community was found to be 2.9 and 0%, respectively.

Serum antibodies to human T-cell leukemia/lymphoma virus I (HTLV-I) were observed in two mothers of the six studied (33.3%). A grandmother, the mother of one of the anti-HTLV-I-positive CHS mothers, also showed anti-HTLV-I antibody. This frequency of 33.3% in known heterozygous subjects is high compared to the overall frequency of 4.2% in the State of Táchira (21). No serum antibodies to this viral antigen have been detected in CHS patients.

Serum antibodies to parietal cells were detected in 37.5% of the mothers and 25% of the fathers. Anti-smooth muscle antibodies were observed in 44.4% of the mothers and 20% of the fathers. Antibodies to nuclear antigens, thyroid microsome, mitochondria, or rheumatoid factor were not detected in mothers or fathers (Table IV). The percentage positivity observed in the CHS heterozygotes is high compared to the frequency for normal control populations (<1%). Cytotoxins reactive with more than 20% of the T- and B-lymphocyte panel were found in five of eight mothers' sera, while at least 2 of 15 B-lymphocyte panel cells were killed by cytotoxins in two of the fathers' sera and in one patient (Table V), the incidence of cytotoxins in the general population being less than 1%.

DISCUSSION

Reports in the literature have indicated the existence of cell alterations in known heterozygous subjects for the Chediak–Higashi gene similar to those observed in the homozygous condition, indicative of partial expression of the CHS gene. However, none of the described alterations is characteristic or diagnostic of this genetic condition.

The results reported herein indicate that immunological alterations similar to those seen in CHS patients can be observed in their parents without causing clinical manifestations, i.e., large cytoplasmic azurophilic granules under light microscopy, conspicuous in electron microscopy, lower natural killer-cell activity, and an increased percentage of

Table IV. Presence of Serum Autoantibodies in CHS Gene Heterozygotes

Subject	Serum antibody (no. positive/no. tested) Rheumatoid factor	Anti-nuclear	Anti-thyroid microsome	Anti-mito-chondria	Anti-parietal cells	Anti-smooth muscle
Mothers	0/9	0/9	0/9	0/2	3/8 (37.5%)	4/9 (44.4%)
Fathers	0/6	0/6	0/6	0/2	1/4 (25%)	1/5 (20%)
Patients	0/9	0/9	0/9	0/2	1/7 (14.3%)	2/9 (22.2%)
General population	<1%	<1%	<1%	<1%	<1%	<1%

Table V. Presence of Antilymphocytotoxic Antibodies in Sera from CHS Heterozygotes

	Serum lytic activity[a] of lymphocyte suspension from donor no.														
	B lymphocytes														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mothers															
HdeC	—	—	1	2	1	2	2	2	2	2	—	1	—	—	—
IdeR	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—
MdeR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PdeM	—	—	—	—	—	—	1	1	2	2	—	—	—	—	—
EdeR	1	3	2	3	2	2	2	2	2	2	2	2	2	2	2
EdeL	—	1	—	—	—	—	—	—	—	—	1	2	3	—	2
CdeG	—	—	—	—	—	—	1	—	—	—	—	—	2	—	—
GdeP	2	3	3	3	3	2	2	2	2	2	3	—	—	3	2
Fathers															
DAR	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
JUR	—	—	—	—	—	—	—	—	—	—	1	2	2	—	—
LRI	—	—	—	—	—	—	—	1	—	2	—	—	—	—	—
JBT	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
HCO	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—

Table V. Continued.

	Serum lytic activity[a] of lymphocyte suspension from donor no.														
	T lymphocytes														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mothers															
HdeC	—	—	—	—	—	1	2	2	1	—	—	—	—	—	—
IdeR	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
MdeR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PdeM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
EdeR	—	2	3	1	1	—	3	—	—	—	3	2	2	2	3
EdeL	—	—	—	—	—	2	1	—	—	1	—	—	—	—	—
CdeG	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GdeP	—	3	3	3	2	2	3	3	3	1	—	—	—	—	—
Fathers															
DAR	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—
JUR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LRI	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
JBT	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
HCO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a]Lysis score: (—) no reaction; (1) 40–60% lysis; (2) 60–80% lysis; (3) >80% lysis.

OKT8 (suppressor/cytotoxic)-positive cells. Lectin-mediated cytotoxicity was normal in the parents, as opposed to the abnormalities seen in the patients (6). Elevated antibody titers to Epstein–Barr virus with an unusual serological profile similar to that seen in the CHS patients and a higher frequency of antibodies to human T-cell leukemia/lymphoma virus (HTLV-I) were also observed. The antibody response to common viral infections has been previously described as normal (5). These alterations occurred more frequently in mothers than in fathers, which indicates that the expression of the CHS gene might be sex influenced. A possible linkage of this gene to the HLA system has been recently described (33). The possible relation of the observed immunological abnormalities to HLA antigens in the CHS gene carriers is an intriguing speculation.

Serum antibodies to HTLV-I were found at a very high frequency in the CHS patients' mothers. However, no such antibodies have been observed in CHS patients, probably because of the age. Whether the CHS gene determines a high sensitivity to this oncoretrovirus infection remains speculative. However, the significance of the silent viral infection in normal tropical populations is still ill defined (21).

The observation of a high frequency of serum

autoantibodies was an unexpected finding. The majority of lymphocytotoxins reported here was found in mothers' sera and could be explained by sensitization due to previous pregnancies (34). Nevertheless, B lymphocytotoxins were demonstrated with at least two of the panel B cells in two of the fathers. The significance of this observation remains speculative and it is possible that these autoantibodies are related to the EBV chronic persistent infection seen in these CHS gene carriers.

The presence in CHS relatives of large cytoplasmic granules in polymorphonuclear neutrophils, eosinophils, and lymphocytes, identical to those diagnostic of the Chediak–Higashi syndrome, has been described by several authors (10, 22–26). However, it is agreed that their presence does not predict the CHS carrier state. Abnormally large granules, lysosomes, were detected in long-term lymphoblastoid cell lines derived from peripheral blood cells of a CHS father (27). Similarly, it was described that as in homozygotes, lymphoblastoid cell lines from a CHS father contained abnormally large Golgi zones with numerous electron-dense single-bound elliptical structures, and 5–10% of the cells contained single membrane-bound organelles with a concentric lamellated substructure (28). Abnormal cytoplasmic inclusions have also been reported in cultured fibroblasts by some authors (29) but not by others (30).

Decreased chemotactic activity (12), as well as significantly lower values of the activities of acid phosphatase, *N*-acetylglucosaminidase, aryl sulfatase, and β-glucuronidase (13), has been observed in peripheral blood neutrophils from CHS heterozygotes. No changes were found in the enzymatic content of lymphocyte lysosomes.

Skin abnormalities seen in electron microscopic studies (9) and abnormal serum lecithin and sphingomyelin phospholipid fractions (10) have also been described.

We have recently noted the occurrence of Chediak–Higashi syndrome in a black Venezuelan child (31). A study of the relatives indicated alterations in granulocyte function in the parents similar to those in the patient, i.e., decreased neutrophil digestive capacity, decreased chemotactic activity, and increased spontaneous spreading of granulocytes.

We have also recently reported the presence of a novel heterophil antibody in Chediak–Higashi syndrome patients (32). This heterophil antibody was also present in heterozygotes for this disease. The significance of this novel antibody is unclear and its detection occurs without evidence of previous EBV infection.

In summary, the CHS heterozygotes can provide a model to study the cellular immune defects caused by the CHS gene and the immunological mechanisms controlling the normal carrier state of the Epstein–Barr viral infection.

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