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Research Article

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Defective Granulocyte Chemotaxis in the Chediak-Higashi Syndrome

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ABSTRACT In vivo and in vitro studies of granulocyte chemotaxis were performed in three patients with the Chediak-Higashi syndrome. Rebuck skin windows showed a decreased accumulation of leukocytes at an inflammatory site. Studies in Boyden chambers documented a cellular defect in granulocyte chemotaxis. The chemotactic response of Chediak-Higashi cells by this technique averaged approximately 40% of normal and was consistently reduced using several different chemotactic stimuli. This deficit was magnified by shortening the chamber incubation time or by decreasing the pore size of the micropore filter and was independent of granulocytopenia. No abnormalities of passive motility, adhesiveness, viability, or pH optimum for migration were found in these cells. Chediak-Higashi serum contained no inhibitors of chemotaxis and was capable of generating normal amounts of chemotactic factors with the exception of one patient with the accelerated phase of the disease. Heterozygotes for the Chediak-Higashi trait had normal chemotactic function. This cellular defect in chemotaxis may contribute to the marked susceptibility to pyogenic infections which is so characteristic of patients with the Chediak-Higashi syndrome.

INTRODUCTION

The Chediak-Higashi syndrome (CHS)[1] is a rare disorder, inherited as an autosomal recessive trait, and characterized by recurrent and severe pyogenic infections (1), partial oculocutaneous albinism, and giant cytoplasmic granules found in a number of cell types. The giant granules are most apparent in peripheral blood leukocytes and have many of the characteristics of lysosomes (2). Animal models of CHS have been described in mink (3, 4), cattle (4), and mice (5–7).

Studies of host resistance to infection in this syndrome have centered primarily on leukocyte regulation and function. Granulocytopenia is a consistent finding in humans with CHS, and studies of granulocyte mobilization have documented decreased marrow granulocyte reserves in spite of adequate numbers of precursors in the marrow (8). Granulocyte responses to infection are likewise diminished.[2] Leukocyte ingestion of bacteria and inert particles is normal in humans (9–13) as well as mink and cattle with CHS (14). Abnormalities in degranulation, associated in some studies with defective intracellular bactericidal activity have been reported (12–16). Abnormal distribution and reduced activity of several lysosomal enzymes have been described (17). Passive leukocyte motility (10, 18) and migration of granulocytes into the peritoneal cavity of CHS mink and cattle (14) are quantitatively normal.

The study of leukocyte chemotaxis has been greatly facilitated by the Boyden chamber technique (19), although clinical applications of this method have been limited. Cellular defects in chemotaxis have been described in "toxic" neutrophils from patients with severe acute infections (20), in two children with recurrent infections and a number of leukocyte abnormalities (21), as an isolated leukocyte defect in a child with recurrent infections (22), and by a somewhat different technique, in patients with diabetes mellitus and rheumatoid arthritis (23, 24). A few cases have been reported in which there were serum defects in generation of chemotactic factors (25, 26) and, in a single case, a serum inhibitor of chemotaxis (27). In the present report, a defect in in vitro granulocyte chemotaxis associated with abnor-

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CHS, Chediak-Higashi syndrome; HPF, high-power field.

[2] Blume, R. S., and S. M. Wolff. Manuscript in preparation.

mal in vivo leukocyte migration is described in three patients with the Chediak-Higashi syndrome.

METHODS

Patients

Since some of the clinical features of the three CHS patients studied have been published elsewhere (28, 29) and detailed case histories are in preparation for publication,[2] only very brief summaries are given here. Patient 1 (Le. R.) and patient 2 (La. R.) are 20 and 19 yr old brothers with histories of recurrent infections since early childhood. Subcutaneous abscesses usually caused by *Staphylococcus aureus* have been the most frequent problem, but respiratory infections, sinusitis, and other types of infections have also occurred. Typical giant intraleukocyte granules and partial albinism are present in both patients. Current therapy is limited to appropriate antibiotics when infections occur. Patient 3 (T. H.) is a 9 yr old girl with the typical findings of CHS and a lifelong history of recurrent infections including staphylococcal skin abscesses, chronic sinusitis, mastoiditis, otitis media, conjunctivitis, rhinitis, impetigo, and pneumonia. At age 5, she developed the accelerated lymphoma-like stage of CHS with severe hepatosplenomegaly and pancytopenia. Treatment with 1.2 mg of vincristine (i.v.) every 3–6 wk has resulted in clinical improvement. In the present studies leukocytes were examined at least 3 wk after the last dose of vincristine. None of the three patients was studied during, or for at least 3 wk after, an acute infection.

Control subjects were selected from healthy laboratory personnel of both sexes ranging in age from 18 to 35 yr and from patients with a variety of inflammatory and infectious diseases.

Skin window migration

Skin window migration was determined according to the method of Rebuck and Crowley (30). Migration was evaluated at 1, 3, 5, 7, 9, 12, and 24 hr, and the magnitude of the cellular response was graded as follows: 0 = less than 10 cells; 1 = 10–50 cells; 2 = 50–150 cells; 3 = 150–400 cells; and 4 = greater than 400 cells on the entire slide. The relative numbers of polymorphonuclear leukocytes and mononuclear cells were also determined. The counting was performed in blinded fashion by two independent observers.

Chemotaxis

Cell preparations. Peripheral blood was drawn in heparin (40 U/ml) and after diluting in an equal volume of 3% dextran (Dextran T250, Pharmacia, Uppsala, Sweden) in 0.85% saline was allowed to sediment at 37°C for 20 min. The supernate was collected and centrifuged at 250 *g* at 8°C for 10 min. Residual erythrocytes in the cell button were lysed by suspending the cells in 0.2% saline for 20 sec, followed by dilution with an equal volume of 1.6% saline. The cells were centrifuged again and washed in Gey's balanced salt solution pH 7.25 with 2% bovine serum albumin (BSA), penicillin, and streptomycin added (Gey's medium; Microbiological Associates, Inc., Bethesda, Md.). After centrifuging the cells were resuspended in Gey's medium with 2% BSA, quantitative and differential cell counts were performed, and the volume of the cell suspension was adjusted to contain 2.3×10^6 granulocytes/ml.

Chemotactic stimuli. Blood was allowed to clot at room temperature for 1 hr. After centrifuging at 3000 *g* for 10 min, the serum was removed and either used immediately or stored at −70°C for future use. Fresh or fresh frozen serum (0.05 ml) was added to 0.1 ml of a 300 μg/ml solution of endotoxin in 0.85% saline (*E. coli* 0127:B8 lipopolysaccharide B; Difco Labs, Detroit, Mich.) and mixed with 0.85 ml of gelatin-Veronal-buffered saline supplemented with Mg^{++} and Ca^{++} cations. Chemotactic factor was generated by incubating this mixture at 37°C for 60 min, and residual complement was then inactivated by heating at 56°C for an additional 30 min. This mixture was diluted in 1.0 ml of Gey's medium with 2% BSA before use in the chemotaxis chamber. In some studies 0.1 ml of sterile fresh frozen guinea pig serum (Suburban Serum Laboratories, Bethesda, Md.) was used in place of human serum. A serum-free bacterial culture filtrate (31) kindly supplied by Dr. Thomas Tempel was also used as the chemotactic stimulus.

Chemotaxis chambers. Chemotaxis chambers consisted of an upper and lower compartment separated by a 5 μ micropore filter (Millipore Corp., Bedford, Mass.). The chemotactic stimulus (2.0 ml) was placed in the lower compartment, and the cell suspension (0.8 ml containing 1.84×10^6 granulocytes) was placed in the upper compartment. The chambers were incubated at 37°C with 5% carbon dioxide and 100% humidity for exactly 3 hr. In some studies incubation time was varied according to experimental design. The filters were then removed, fixed in methanol, stained with hematoxylin, dried in ethanol, and cleared in xylene.

Stained filters were examined at a magnification of 550 × and the number of cells which had migrated through to the lower surface of the filter was counted in 5–10 random fields. Chemotactic activity was expressed as the average number of cells per high-power field (HPF). The chemotactic response in each individual experiment was taken as the average of four duplicate chamber values. The means of these responses for a number of separate experiments were compared using the standard two sample *t* test. Standard error is used throughout as an estimate of variance.

RESULTS

Skin window migration

Table I shows the average cellularity score and percentage of granulocytes for each time period in nine skin windows on the three patients with CHS and five in normal subjects. The data were analyzed by calculating a mean score for each individual study over the entire 24 hr time period (normal 3.1 ±0.2, CHS 2.2 ±0.3) and by a rank sum test (Friedman test with partitioning of the Chi square [32, 33]). Both methods showed a significant reduction in the accumulation of cells in the CHS patients ($P < 0.05$). This deficit was most pronounced for granulocytes, but the later mononuclear phase also appeared to be reduced. The relative numbers of granulocytes and mononuclear cells in the CHS preparations were similar to normal.

Studies of CHS cell chemotaxis

In each experiment, cells from one or more CHS patients were studied concomitantly with cells from one or more normal subjects. The chemotactic stimulus in the lower compartment was generated from both autologous

TABLE I

Rebuck Skin Window Responses in CHS Patients

Subjects		Time in hours 1	3	5	7	9	12	24
Normal (5)	Cellularity*	1.1‡	2.1	3.4	3.6	3.6	3.6	4.0
	Granulocytes, %	99	94	93	71	47	45	16
CHS (9)	Cellularity	0.2	1.0	1.8	3.1	2.3	2.3	2.8
	Granulocytes, %	—§	95	86	68	61	39	13

* See text for explanation.

‡ Geometric mean.

§ Too few cells to count.

CHS and autologous normal serum by incubation with endotoxin. Background levels of migration were determined by omitting the serum and were in the range from 5 to 20 cells/HPF.

The results of individual experiments in each CHS patient are shown in Fig. 1. Decreased chemotaxis of CHS granulocytes is clearly apparent in each of these patients regardless of whether the chemotactic stimulus was generated from normal serum or CHS serum. The sera of patients Le. R. and La. R. were comparable with normal serum in their ability to generate chemotactic activity for either normal or autologous CHS cells. In contrast, the serum of patient T. H. was reduced from normal in its ability to generate chemotactic activity and will be dealt with in more detail later. The results of all 28 experiments with CHS and normal cells are summarized in Table II. When chemotactic factor was generated from normal human serum, a significant and consistent deficit was found in CHS leukocyte chemotaxis and was of equal magnitude in the three patients (mean 41.2% of normal). A similar defect was noted in CHS cells when the stimulus was generated from CHS serum, although the magnitude of the defect varied somewhat among the sera used.

The patients in this series had persistent granulocytopenia (average total granulocyte count of 1700/mm^3). Although dextran sedimentation resulted in an enrichment of the percentage of neutrophils, the CHS cell preparations had consistently lower percentages of neutrophils than the normal cell suspensions (mean normal 75.9% ±1.9, mean CHS 45.3% ±3.5). Thus, although the final concentrations of neutrophils in CHS and normal cell suspensions were equalized (2.3×10^6/ml), the CHS preparations contained a higher percentage of contaminating mononuclear cells. Three lines of evidence suggest that these contaminating cells have no appreciable effect on granulocyte chemotaxis. First, differential counts of the cells which had migrated through the filter showed that in both normals and CHS patients, the migrating cells were at least 90% neutrophils. Second, using purified granulocyte suspensions (96% in normal and 92% in CHS) prepared by differ-

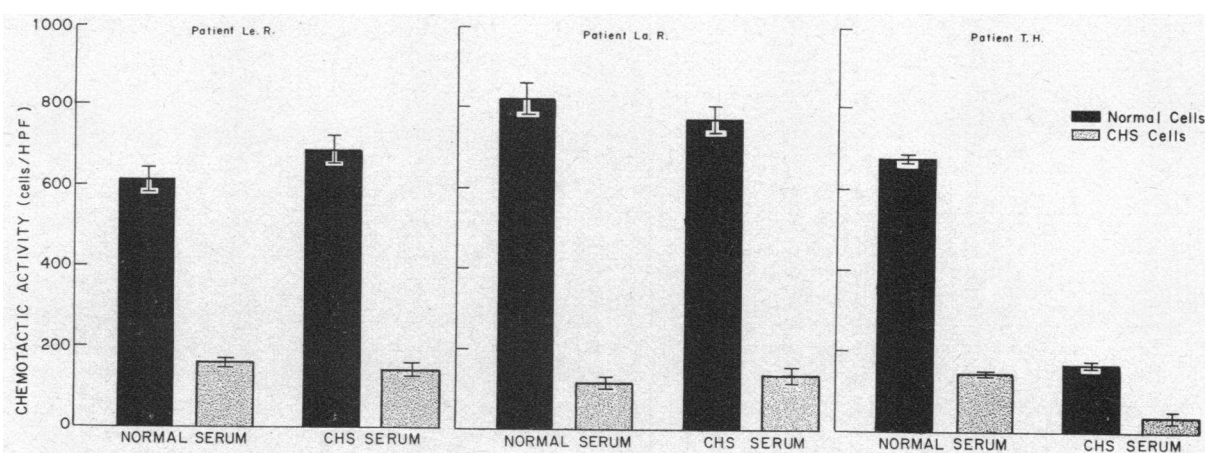

FIGURE 1 Granulocyte chemotaxis (mean ±SE) in three patients with Chediak-Higashi syndrome. In each experiment the chemotactic stimulus was generated by endotoxin activation of autologous normal and autologous CHS serum.

TABLE II

Chemotaxis of CHS Granulocytes

Serum source*	Granulocytes	Chemotaxis	% of normal	P‡
Normal	Normal	609 ±39 (14)§	100	—
	CHS			
	Le. R.	242 ±37 (6)	39.7	<0.01
	La. R.	252 ±75 (7)	41.4	<0.01
	T. H.	264 ±80 (4)	43.3	<0.01
	All CHS	251 ±36 (17)	41.2	<0.01
Le. R.	Normal	634 ±85 (3)	100	—
	CHS	360 ±109 (3)	56.8	<0.2
La. R.	Normal	696 ±26 (4)	100	—
	CHS	288 ±81 (4)	41.4	<0.01
T. H.	Normal	264 ±72 (4)	100	—
	CHS	46 ±5 (4)	17.4	<0.05

* Serum used for generation of chemotactic stimulus.
‡ Significance level of difference from normal.
§ Cells/HPF, mean ±SE; number of experiments in parenthesis.

ential density centrifugation in a hypaque-ficoll density gradient (34), the chemotactic deficit persisted (normal 753 ±67; CHS 296 ±29 or 39.3%). Third, the chemotactic activity of normal dextran-sedimented granulocytes was not affected by mixing them in a 1:1 proportion with 98% pure CHS mononuclear cells from a hypaque-ficoll gradient (normal granulocytes 451 ±42; CHS mononuclear cells 2 ±1; 1:1 mixture 407 ±99). The mononuclear preparation contained monocytes and lymphocytes in a ratio of 1:4, a proportion approximating that of the mononuclear contamination in the dextran preparations.

Other chemotactic stimuli (Table III). An appreciable amount of chemotactic activity is present when fresh human serum is incubated at 37°C without endotoxin (35). With this nonactivated serum chemotactic stimulus, the mean response of CHS cells was 32.0% of normal. The chemotactic response of CHS cells was also reduced when endotoxin activated guinea pig serum or a serum-free bacterial culture filtrate was used (63.8% and 38.3% of normal, respectively).

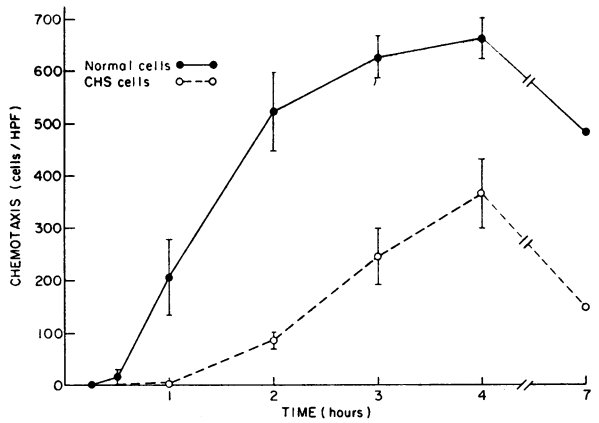

FIGURE 2 Time course of granulocyte chemotaxis (mean ±SE) in six normal individuals and three CHS patients (eight studies). The chemotactic stimulus was endotoxin-activated normal human serum.

Kinetics of cell migration. The results described thus far were all obtained using a 3 hr migration period in the chemotaxis chambers. In additional studies, the time course of granulocyte migration was examined at times varying from 15 min to 7 hr (Fig. 2). The defect in CHS cell migration was found throughout, but was most pronounced at the earlier time periods (1.0% of normal at 1 hr, 16.3% at 2 hr, 39.3% at 3 hr, and 55.3% at 4 hr). After 4 hr of incubation, cell counts in both normal and CHS patients diminished considerably probably due to increasing cell death.

Kinetics of granulocyte chemotaxis were studied in three subjects heterozygous for CHS (mother and father of Le. R and La. R. and father of T. H.). At time periods from 1 to 4 hr, the chemotactic responses of cells from heterozygotes were normal.

TABLE III

Granulocyte Response to Various Chemotactic Stimuli in CHS

Stimulus	Chemotaxis			
	Normal cells	CHS cells	% of normal	P*
Endotoxin-activated normal human serum	609 ±39 (14)‡	251 ±36 (17)	41.2	<0.01
Nonactivated normal human serum	231 ±32 (10)	74 ±13 (8)	32.0	<0.01
Endotoxin-activated guinea pig serum	229 ±28 (9)	146 ±25 (10)	63.8	<0.05
Bacterial culture filtrate§	530 ±27 (1)	203 ±17 (1)	38.3	<0.01

* Significance level of difference from normal.
‡ Cells/HPF, mean ±SE; number of experiments in parenthesis.
§ See text for explanation.

Effect of pore size, pH, and serum on cell migration. As expected, decreasing the pore size of the micropore filter used in the chemotaxis chamber from 5 μ to 1.2 μ resulted in considerably less migration. However, CHS cell chemotaxis was only 9.5% of normal with the 1.2 μ filter (mean of three experiments) as compared with the previously noted figure of 41.2% with the 5 μ pore size. To examine the possibility that CHS cells have a different pH optimum for migration, experiments were done at pH 7.00, 7.25, and 7.50. These changes in pH had no significant effect on the chemotactic activity of either normal or CHS cells (normal 517, 470, 503; CHS 207, 201, 200, respectively), and the magnitude of the chemotactic defect in CHS cells remained constant.

Direct inhibitors of cell migration were searched for by adding 10% normal or CHS serum to normal cell suspensions in the upper compartment of the chamber (27). Apparent inhibition (approximately 80%) was seen with all sera so tested. This is probably explained by the fact that the serum in the upper compartment of the chamber has appreciable intrinsic chemotactic activity and, therefore, reduces the gradient of chemotactic factors across the micropore filter resulting in less migration (19). In no case was the reduction in cell migration any greater with CHS serum than with normal serum.

Viability and passive motility. Viability was examined by trypan blue exclusion in cells removed from the upper compartment of the chemotaxis chamber at the end of the 3 hr incubation period. In four preparations each of normal and CHS cells, an average of 96% and 94% of cells, respectively, was considered to be viable by this technique. Passive motility and adhesiveness of CHS cells were normal as examined by the capillary tube migration technique (36, 37).

TABLE IV

Granulocyte Chemotaxis in Patients with a Variety of Diseases

Diagnosis	Chemotaxis
	% of normal control
Systemic lupus erythematosis (five patients)	80
	90
	101
	108
	86
Recurrent bacterial infections without specific diagnosis (three patients)	137
	90
	96
Sarcoidosis	105
Chronic glomerulonephritis with uremia	92
Miliary tuberculosis	81
South American blastomycosis	123
Systemic vasculitis	167
Chronic mucocutaneous moniliasis	135
Familial Mediterranean fever	100
Lethal midline granuloma	88
Malignant lymphoma	90
All patients (mean ±SE)	104 ±5.7

TABLE V

Chemotactic Activity of Endotoxin-Activated Normal and CHS Serum

Serum	Test cells	Chemotaxis	% of normal	P*
Normal	Normal	635 ±43 (13)‡	100	—
CHS				
Le. R		634 ±85 (3)	99.8	>0.9
La. R		696 ±26 (4)	109.6	>0.4
T. H.		218 ±36 (9)	34.3	<0.01
Normal	CHS	271 ±48 (10)	100	—
CHS				
Le. R.		360 ±109 (3)	132.8	>0.4
La. R.		288 ±81 (4)	106.3	>0.8
T. H.		46 ±5 (4)	17.0	<0.02

* Significance level of difference from normal.

‡ Cells/HPF, mean ±SE; number of experiments in parenthesis.

Specificity. The CHS patients studied had a history of frequent infections, and although none was acutely infected when these studies were performed, the diminished chemotaxis observed might have been a nonspecific event accompanying inflammation or repeated infections. Accordingly, studies of chemotaxis were performed on a total of 17 patients with a variety of infectious, inflammatory, or neoplastic diseases (Table IV). In none of these subjects was granulocyte chemotaxis significantly reduced from normal controls.

Studies of CHS serum

Serum from each of the three CHS patients was tested for its ability to generate chemotactic activity on incubation with endotoxin (Table V). While sera from Le. R. and La. R. were both normal, serum from T. H. was consistently deficient when tested with either normal cells (34.3% of normal) or CHS cells (17.0% of normal). Likewise, T. H. serum was also less potent in its intrinsic chemotactic activity present without activation by endotoxin or other agents (mean normal activity 231 ±32 in 10 experiments; mean T. H. activity 111 ±38 in 6 experiments, 48.1% of normal, $P < 0.05$). Serum from one CHS heterozygote, the father of T. H., was normal in its ability to generate chemotactic activity.

Since the chemotactic factor produced in both human

and guinea pig serum by endotoxin activation is an active fragment of the fifth component of complement (38, 39), serum complement levels were performed in patient T. H. Total hemolytic complement levels were normal (59 and 60 hemolytic units, normal 35–60). C'3 measured by immunodiffusion was 163 mg/100 ml (normal 119–175). Hypogammaglobulinemia, a common feature in the accelerated phase of CHS, was present in T. H. (IgG 200 mg/100 ml). Addition of a commercial preparation of IgG (Fraction II; Pentex Biochemical, Kankakee, Ill.) to T. H. serum in vitro failed to restore normal chemotactic activity. When increasing amounts of T. H. serum were tested, progressive increases in chemotactic activity were found. Small amounts of normal serum added to T. H. serum failed to restore normal activity. The chemotactic activity of normal serum was enhanced in an additive manner when mixed in ratios of 100:1, 10:1, and 1:1 with T. H. serum suggesting that inhibitors were not present.

DISCUSSION

The purpose of these studies was to determine whether granulocytes from patients with CHS are capable of normal chemotactic responses. Leukocyte migration in vivo was significantly less than normal in the three patients studied. These findings are at variance with a previous report of normal skin window migration in two patients with CHS (10), but confirms a preliminary study (40). The results in the present report cannot be attributed to drug therapy since two of the patients (Le. R. and La. R.) were receiving no pharmacologic agents and the patient receiving vincristine (T. H.) was studied 3 wk or more after the last dose. Neutropenia might be an explanation for the failure in the early skin window responses. It has been shown, however, that unless total granulocyte counts are below 1500/mm^3, skin window responses are normal (41). Granulocyte counts in the patients in the present study were generally above this level (mean 1700/mm^3). Furthermore, within the group of CHS patients, there was no correlation between individual blood granulocyte counts and mean cellular responses (Spearman rank correlation test [42]). It seems likely, therefore, that an intrinsic cellular defect rather than neutropenia is responsible for the abnormal responses observed.

The in vitro studies of granulocyte chemotaxis utilizing the Boyden chamber technique clearly demonstrate defective CHS cell migration. The defect was present in all three patients studied, was of similar magnitude for each, persisted over a 4 hr time period, and was present when tested with a number of different chemotactic stimuli. The work of other investigators has demonstrated that the chemotactic factors found in endotoxin-activated serum, nonactivated serum, and bacterial culture filtrates are clearly distinct from one another (31, 35, 38, 39, 43–46). This suggests that the CHS defect is not due to a failure in recognition of a specific stimulus.

Further studies suggest that the defect in chemotaxis of CHS granulocytes cannot be ascribed to diminished viability, poor motility, altered pH optimum, mononuclear cell contamination, or serum inhibitors. The fact that granulocyte chemotaxis was normal in a number of patients with a variety of diseases argues against the view that the defective chemotaxis in CHS is a nonspecific host response to inflammation or repeated infections.

The experiments performed with the smaller pore size filters are of particular interest. As noted, the reduction in pore size greatly magnified the defect in CHS cell chemotaxis. If the fundamental defect were in stimulus recognition, then a constant, albeit diminished, proportion of cells should migrate regardless of pore size. It seems more likely, therefore, that the cellular defect involves either the biochemical events responsible for migration or interference with the passage of the cells through the pores of the filter. For example, a limitation in cellular deformability due to an alteration in surface properties or an increase in cytoplasmic rigidity may mechanically interfere with migration. The giant CHS lysosomal granules which are often as large as 4 μ in diameter (47) may be relatively rigid structures and thereby impede the cells' ability to maneuver through the small pores of the chemotaxis chamber filter or to migrate in vivo from the blood to a site of infection. If this is true, the chemotactic defect and the observed failure of granulocyte mobilization (despite adequate numbers of marrow granulocytes and precursors) (8) may have a common basis since studies by Lichtman suggest that leukocyte deformability is important in the egress of cells from the bone marrow (48).

Very little is known about the mechanism of leukocyte responses to chemotactic factors. The finding of an intrinsic cellular defect in CHS may assume added importance by providing a pathologic model for the study of this process. This point is underscored by recent studies in CHS mink which show a cellular defect in chemotaxis entirely comparable with the current findings in humans (18).

In another aspect of the present studies sera from two of the CHS patients were normal in their ability to generate chemotactic activity. The defect in generation of chemotactic factors in the serum of the third patient (T. H.) with the accelerated lymphoma-like phase of CHS remains unexplained. The data suggest that hypoglobulinemia, deficient complement levels, or serum inhibitors cannot be implicated.

Considering the current results in relation to previous studies of CHS, it seems likely that multiple factors including granulocytopenia (8), poor bone marrow granulocyte mobilization (8), decreased leukocyte bactericidal capacity (13, 15, 16), and defective granulocyte chemotaxis are involved in the increased susceptibility to infections characteristic of the Chediak-Higashi syndrome.

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