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*J Clin Invest.* 1982;69(5):1155-1163. <https://doi.org/10.1172/JCI110551>.

### Research Article

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# Mononuclear Cells from Patients with the Hyperimmunoglobulin E-Recurrent Infection Syndrome Produce an Inhibitor of Leukocyte Chemotaxis

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**ABSTRACT** The chemotactic responsiveness of the neutrophils of 10 patients with the hyperimmunoglobulin E-recurrent infection syndrome (HIE) were compared with neutrophils from normal volunteers over a 10-mo period. HIE neutrophils as a group displayed significantly less chemotactic motility than control neutrophils. The data from individual patients were variable, being normal or abnormal on different days. Mononuclear cells from HIE patients, when cultured for 24 h in the absence of serum or a mitogen, produced a factor that inhibited normal neutrophil and monocyte chemotaxis. Mononuclear cells from normal volunteers with and without atopy or from patients with parasites or bacterial infections did not produce such an inhibitory factor. The production of this chemotactic inhibitory factor *in vitro* was variable over time, but it correlated with the presence of an *in vitro* neutrophil chemotactic defect. The chemotactic inhibitory factor was partially purified and was found to contain protein, to be stable at 56°C, and to have a molecular weight of ~61,000. Irreversible inhibitors of serine esterases do not inactivate the factor. The factor is produced by esterase-negative mononuclear cells and is not toxic to neutrophils. This chemotactic inhibitory factor may be the basis of the variable chemotactic defect in HIE neutrophils.

## INTRODUCTION

In 1966, Davis et al. (1) described a disorder of recurrent "cold" abscesses in red-haired girls, which they called "Job's" syndrome. Subsequently, the work of several investigators has enlarged and deepened the understanding of this syndrome (2-5). It has become

apparent that the disease has its onset early in life and can afflict both black and white patients of either sex. It is characterized by recurrent bacterial infections of the skin and sinopulmonary tract. These infections are often remarkable for their paucity of surrounding inflammation, causing so-called "cold" abscesses. The infections are frequently caused by *Staphylococcus aureus*, although other pathogens such as *Hemophilus influenzae* can be involved. Approximately 50% of hyperimmunoglobulin E-recurrent infection syndrome (HIE)<sup>1</sup> patients have mucocutaneous candidiasis. These patients have recurrent eczematoid rashes and have a moderate blood eosinophilia, but often lack any increased allergic symptomatology. They have characteristically high immunoglobulin E (IgE) levels that may be twenty times greater than normal (6). The syndrome has been called the hyperimmunoglobulin E-recurrent infection syndrome (HIE).

The basis for recurrent bacterial infection in HIE patients is unknown. HIE patients have abnormal cell-mediated immunity as well as IgE specific for potential pathogens (2, 7). These findings have been suggested to be the basis of the repeated bacterial infections. We believe that a deficiency in polymorphonuclear leukocyte (PMN) function may be more important in the pathogenesis of the repeated infections. Studies in several laboratories (2, 4, 5) and in ours revealed that, although HIE patients are not neutropenic and their PMN ingest and kill bacteria normally, both their neutrophils and monocytes have a chemotactic defect *in vitro* (3-6, 8-12). But as Buckley & Becker (6) pointed

Received for publication 6 August 1981 and in revised form 18 January 1982.

Presented in part at the American Federation of Clinical Research Annual Meeting, San Francisco, 27 April 1981.

<sup>1</sup> Abbreviations used in this paper: CGD, chronic granulomatous disease; DFP, diisopropyl fluorophosphate; HIE, hyperimmunoglobulin E-recurrent infection syndrome; IgE, immunoglobulin E; LIF, leukocyte inhibitory factor; PMN, polymorphonuclear leukocyte; PMSF, phenylmethyl sulfonyl fluoride.

out, the chemotactic defect of HIE leukocytes is not constantly present in these patients.

We felt that an abnormality in the immunoregulatory system could explain the peculiar findings of the HIE syndrome. We hypothesized the presence of an immunoregulatory defect in the mononuclear cells of HIE patients that would cause an increased production of IgE to many antigens as well as cause the production of an inhibitor of phagocytic cell chemotaxis. This paper describes the discovery and partial characterization of a chemotactic inhibitory factor produced by HIE mononuclear cells. We also correlate the presence of this factor and the finding of a chemotactic abnormality *in vitro*.

## METHODS

**Patients.** 10 patients with HIE who ranged in age from 6 to 52 yr were studied at the National Institutes of Health (NIH). They were diagnosed as having HIE if they had a history of recurrent sinopulmonary infections starting in infancy or childhood and had elevated serum IgE levels in the absence of another cause for these findings. All of the patients studied were female: two were black and the rest were white. They all had elevated IgE levels averaging  $13,690 \pm 3,536$  (range of 500 to 49,000;  $N < 200$  IU/ml). Five patients had recurrent candidiasis. The patients were seen at the NIH for routine followup as well as for many infectious complications of their disease. 8 of the 10 HIE patients have been entered in a prospective double-blind trial testing the clinical efficacy of the investigational drug, levamisole. This study calls for the patient to take levamisole (50 or 100 mg) or placebo orally twice a week for 4-mo periods, allowing several weeks between the 4-mo test periods for patient evaluation. Preliminary studies indicated that any effect of levamisole on mononuclear cell cultures administered in this manner disappeared 10 d after the last dose of levamisole. No patient evaluated in this paper received levamisole  $< 10$  d before his evaluation.

Normal volunteers were employees of the NIH, were of both sexes, and were both black and white. Atopic and infected patients were drawn from the NIH inpatient population. Consent was obtained for the use of their blood in these studies.

**Materials.** The following were obtained: RPMI 1640 with glutamine, pokeweed mitogen, and Hanks' balanced salt solution without phenol red, with or without calcium and magnesium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY); Gey's solution with 2% bovine serum albumin (Microbiological Associates, Walkersville, MD); N-formyl methionylleucylphenylalanine (f-methionine-leucine-phenylalanine, f-Met-Leu-Phe) (Peninsula Labs, San Diego, CA); f-Met-Leu- $^3$ H]Phe (55.6 Ci/mmol) (New England Nuclear, Boston, MA); NCS tissue solubilizer (Amersham, Arlington Heights, IL); 3a20 scintillation cocktail (Research Products International Corp., Mt. Prospect, IL); gentamicin (10 mg/ml) (Schering, Kenilworth, NJ); Phenylmethyl sulfonyl fluoride (PMSF), trypsin and papain (Sigma Chemical Co., St. Louis, MO); Diisopropyl fluorophosphate sodium (DFP) (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA); Sodium caseinate (Difco Laboratories, Detroit, MI), nitrocellulose 3- $\mu$ m pore size chemotaxis filters (Sartorius, Göttingen, West Germany).

**Culture of mononuclear cells.** Heparinized venous blood

was obtained from HIE patients and normal volunteers. Mononuclear cell and PMN fractions were separated on Hypaque-Ficoll gradients as described (13, 14). Mononuclear cells thus obtained were  $>97\%$  pure and contained  $\sim 85\%$  lymphocytes and 14% monocytes by morphological characteristics. There was no difference between normal and HIE patients' mononuclear cell composition of monocytes and lymphocytes. The mononuclear cells were washed twice in Hanks' solution and suspended in RPMI 1640 with glutamine and 10 mg/ml gentamicin at  $5 \times 10^6$ /ml. In early experiments 10% AB serum with and without a 1:1,000 dilution of pokeweed mitogen were added, but these were subsequently deleted because it was found that the difference between normal and HIE mononuclear cells was evident with or without serum and mitogen. The mononuclear cell suspensions were incubated for 24 to 96 h at 37°C with 5% CO<sub>2</sub>, 100% humidity, in polypropylene tubes (12  $\times$  75 mm) without shaking. At the end of the incubation period, the tubes were centrifuged at 1,000 g, and the supernatant was decanted.

**Leukocyte chemotaxis assay.** Neutrophils from normal volunteers were isolated using the cited Hypaque-Ficoll technique (13) and were suspended at  $2.3 \times 10^6$ /ml in Gey's solution with 2% bovine serum albumin whose pH was corrected to 7.4 with NaOH. The neutrophil suspension was placed in the upper compartment of a Boyden chamber containing a 3- $\mu$ m pore size nitrocellulose filter, 180  $\mu$ m in thickness. The lower compartment of the Boyden chamber was filled with 5% endotoxin-activated serum in Gey's solution, 10 nM f-Met-Leu-Phe in Gey's solution, or 5 mg/dl sodium caseinate in normal saline. Some experiments used partially purified C5a as a chemoattractant (15). The morphologic assay of chemotaxis described by Zigmond and Hirsch (16) was used with a 45-min incubation period at 37°C. To facilitate the counting of the filters, a Zeiss Photomicroscope 2 (Carl Zeiss, Inc., New York) was connected to an image analyzer (Optomax CPU-2, Micromeritics, Cambridge, England) interfaced with a Hewlett-Packard 9815 calculator and 7225A plotter (Hewlett-Packard Co., Palo Alto, CA). Two cores with  $1.16 \times 10^5$   $\mu$ m<sup>2</sup> cross sections in each nitrocellulose filter were assessed, with measurements made every 10  $\mu$ m into the filter. Migration was quantitated as either the average number of PMN that migrated 10  $\mu$ m or more into the filter or as the mean distance travelled by the PMN into the filter as described (17).

Mononuclear cells prepared as above were suspended in Gey's with 2% bovine serum albumin at  $3 \times 10^6$ /ml. Chemotaxis was assayed in a 48-well chemotactic chamber using a 5- $\mu$ m pore polycarbonate filter, 12  $\mu$ m in thickness, as described (18). After incubation for 1 h at 37°C, the cells that traversed the polycarbonate filter were counted using the image analyzer apparatus. Duplicate readings from each of four replicate wells were averaged. The mean  $\pm$  SEM of four replicate wells was obtained for each experiment.

**Assay of chemotactic inhibitory activity.** Dilutions of mononuclear cell culture supernatants were added to suspensions of neutrophils at  $2.3 \times 10^6$ /ml or mononuclear cells at  $3 \times 10^6$ /ml in Hanks' without phenol red. The mixtures were incubated with shaking at 37°C for 30 min. The cells were washed twice with cold phosphate-buffered saline. The cells were made up to their original concentration in Gey's, and their chemotactic activity was tested as above. Cells treated with Hanks' alone or dilutions of RPMI 1640 were used as controls. The approximate percent loss of cells during the pretreatment and washing steps was 25%, with no significant difference between control and experimental cells.

The percent inhibition of neutrophil chemotaxis is des-

cribed by the formula:  $100\% \times [1 - (\text{mean distance traveled by experimental cells} / \text{mean distance traveled by control cells})]$ .

The percent inhibition of monocyte chemotaxis is described by:  $100\% \times [1 - (\text{number of experimental cells traversing filter} / \text{number of control cells traversing filter})]$ .

**F-Met-Leu-<sup>3</sup>H]Phe binding assay.** Neutrophils at  $5 \times 10^6/\text{ml}$  in Hanks' solution without calcium or magnesium were exposed to 100 nM f-Met-Leu-<sup>3</sup>H]Phe for 1 h with or without a 1,000-fold excess of unlabeled f-Met-Leu-Phe as described (15). All assays were done on ice. The specific saturable binding is defined as the binding in the absence of excess unlabeled peptide minus the total binding in the presence of unlabeled peptide. The displaceable bound f-Met-Leu-<sup>3</sup>H]Phe was determined by addition of a 1,000-fold excess of unlabeled peptide to neutrophils that had been exposed to 100 nM f-Met-Leu-<sup>3</sup>H]Phe for 1 h on ice. The difference between these two determinations is defined as the displaceable binding. The nondisplaceable binding is defined as the specific binding minus the displaceable binding. 0.5-ml aliquots of neutrophils were layered on silicone oil and centrifuged at 10,000 *g*. The cell pellets were solubilized overnight in NCS tissue solubilizer and the counts per minute were measured as reported (18).

**Gel filtration.** 100  $\times$  2.5-cm G-75 and G-100 Sephadex columns were equilibrated with 0.04 M, pH 7.4, phosphate-buffered normal saline at 4°C. The columns were calibrated with standard molecular weight marker proteins. Supernatant volumes  $\leq 2\%$  of column volumes were layered on the gel. The elution rate was  $\sim 40$  ml/h with the phosphate-buffered saline buffer as the eluant. Fractions of 6.3 ml were collected and assayed for chemotactic inhibitory activity.

**Bactericidal assay.** Buffer and chemotactic inhibitory factor-exposed PMN were exposed to a 50:1 excess of *Staphylococcus aureus* opsonized with fresh human serum. The number of living staphylococci at 20, 45, and 90 min was determined as reported (19).

**Enzyme assays.** Lysozyme and lactate dehydrogenase enzyme assays were performed as described (20, 21).

**Statistics.** The  $\chi^2$  test, paired sample *t* test, Student's *t* test using summary data, Fisher's exact test, and a two-way analysis of variance (patients vs. stimuli) were used to analyze the data as indicated (22), and the width of the largest gap in the distribution of data was tested by a method described by Wilks (23).

## RESULTS

**Presence of a chemotactic defect in HIE patients' neutrophils.** Fig. 1 displays the results of chemotactic assays performed on the neutrophils of 10 different HIE patients over a time period of 10 mo. The results of chemotactic assays on three patients with Chédiak-Higashi Syndrome, a well accepted example of a neutrophil chemotactic defect, are included to demonstrate the variability of the neutrophil chemotactic defect in HIE.

The presence of a chemotactic defect is statistically significant when analyzed in several ways. The asymmetry of the distribution of HIE neutrophil chemotactic activity in relation to the zero line for normal

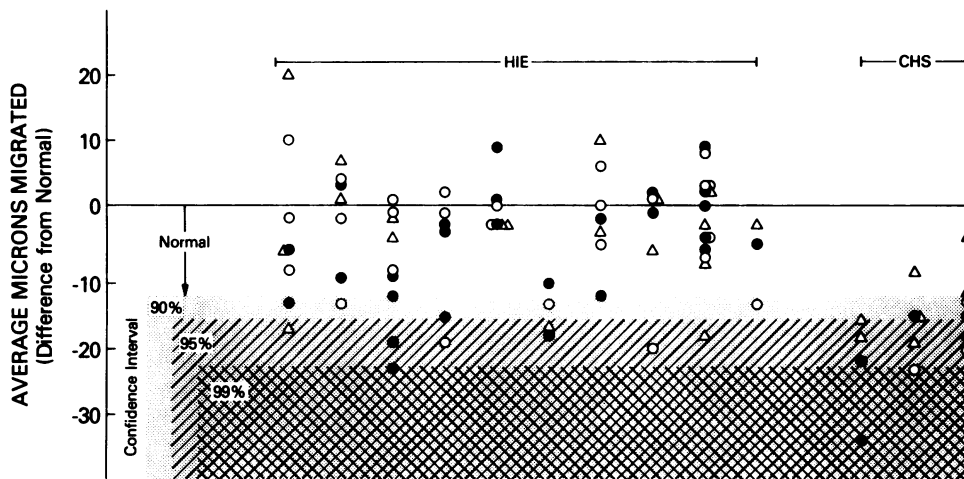


FIGURE 1 Chemotactic responsiveness of HIE and Chédiak-Higashi neutrophils. Each column represents a different patients' responses measured at various times over a 10-mo period. The difference between the average distance migrated of neutrophils obtained from a patient with HIE and a concurrently assayed normal is displayed. A value of 0 indicates that the HIE patient's neutrophils migrated as well as the normals. Values below the zero line indicate less chemotactic motility than the normal. The shaded areas are the confidence bands that represent the 90, 95, and 99% probability of being truly abnormal as determined by the distribution of average distance migrated in 48 determinations from 16 normals. The open and closed circles and triangles represent chemotactic responses to 5% endotoxin-activated AB serum, 5% sodium caseinate in normal saline and 10 nM f-Met-Leu-Phe, respectively. The data from three Chédiak-Higashi patients (CHS) is displayed for comparative purposes. Chemoattractants: ●, *E. coli* endotoxin-activated serum; ○, casein; △, f-Met-Leu-Phe.

neutrophils yields a  $\chi^2$  of 8.55 (df = 1,  $P < 0.01$ ) when the average of each patient's chemotaxis towards three different chemoattractants on each day is analyzed. When the chemotactic responses towards a particular chemotactic factor on each day are taken as independent events and analyzed for asymmetry of distribution with respect to the zero line,  $\chi^2$  equals 11.84 (df = 1,  $P < 0.005$ ). The density of points in the 90% confidence band is also significantly different from the expected distribution of normal neutrophils ( $\chi^2 = 9.35$ , df = 1,  $P < 0.005$ ) when each day's response to a chemoattractant is regarded as an independent event. Analysis of the data by paired sample  $t$  test comparing an HIE patient's neutrophils to a concurrently assayed normal's neutrophils revealed a significantly smaller average distance migrated for HIE neutrophils (40.1  $\mu\text{m}$ ) in relation to normal neutrophils (44.8) using the response to all three different chemotactic factors, casein, f-Met-Leu-Phe, and endotoxin-activated serum ( $n = 71$ ,  $P < 0.001$ ). An analysis of variance performed on this data indicated that the variability of responses to the three different chemoattractants used was smaller than the variability within a patient or among patients ( $P < 0.01$ ). Thus, there was concordance among all three of the chemoattractants used in those studies. HIE neutrophils also had minimal though significantly less random (nondirected) migration when compared with concurrently assayed normal neutrophils (23.9 vs. 26.3  $\mu\text{m}$  average distance migrated for HIE vs. control neutrophils,  $n = 29$ ,  $P < 0.02$ ).

**Presence of an inhibitor of neutrophil chemotaxis.** The chemotactic inhibitory activity of 24-h mononuclear cell culture supernatants is depicted in Fig. 2. The mean percent inhibition of 21 supernatants from 19 normal volunteers was  $4.1 \pm 1.9\%$ . These normals included 7 males and 12 females, of which 5 females and 1 male were atopic by history. The mean percent inhibition for all HIE patients' supernatants was  $19.4 \pm 3.0$  vs.  $4.1 \pm 1.9\%$  for all normals' supernatants ( $P < 0.001$ ). There is a considerable variation in inhibitory activity seen in the HIE patients, and the distribution of values in Fig. 2 appeared bimodal with two clusters of points consistent with no significant production of factor ( $1.4 \pm 2.4\%$  inhibition) and significant production of factor ( $28.4 \pm 1.6\%$  inhibition). The width of the gap between the two apparent distributions was larger than would be expected by chance alone ( $P = 0.056$  for a uniform distribution) suggesting that there was indeed a bimodal distribution of these data. We noticed during the course of these studies that the production of the inhibitory factor in vitro in the same patient over time may cease and then begin again.

The chemotactic factor used in all of these assays for inhibition of chemotaxis and, unless otherwise

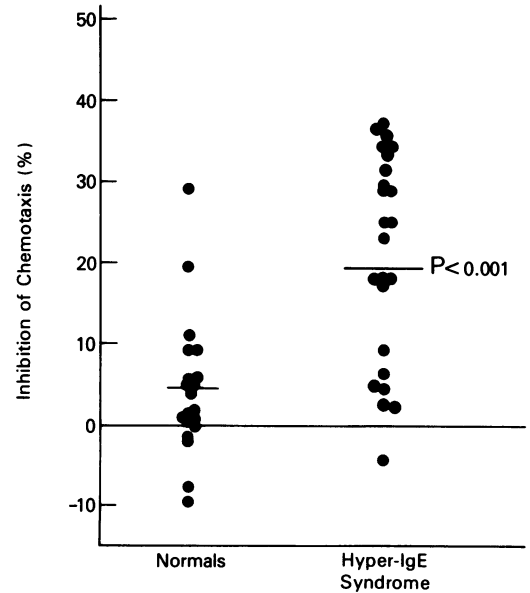


FIGURE 2 The effect of normal and HIE mononuclear cell culture supernatants on normal neutrophil chemotaxis to f-Met-Leu-Phe. The percent inhibition of chemotaxis obtained by treating normal neutrophils with a 1:100 dilution of 24-h mononuclear cell supernatants from normals and HIE patients is displayed. The percent inhibition is calculated as in Methods. Treatment of neutrophils with Hanks' solution represented 0% inhibition.

stated in this paper, was 10 nM f-Met-Leu-Phe. However, similar inhibition of PMN chemotaxis was seen when partially purified C5a or casein was used as a chemotactic factor. In one experiment, an HIE patient supernatant produced 33% inhibition of chemotaxis towards f-Met-Leu-Phe, 35% towards C5a, and 23% towards casein. Thus, the factor does not merely inhibit chemotaxis toward f-Met-Leu-Phe, but to other potent chemoattractants as well. Due to logistical problems, the effect of the inhibitory factor on HIE neutrophils and monocytes was not tested.

The factor was assessed for its effect on neutrophil nondirected migration into nitrocellulose filters. Five experiments were done to evaluate the effect of the factor on nondirected migration of neutrophils. Although a small defect was detected in factor-treated neutrophils, no significant effect could be demonstrated (mean of  $11.0 \pm 7.6\%$  inhibition in all five experiments).

**Concordance between a chemotactic defect and the presence of the chemotactic inhibitory factor.** Because there appeared to be variability in the presence of both the chemotactic defect and the chemotactic inhibitory factor in HIE, a two by two contingency analysis was performed to see if the two findings correlated in the HIE patient studies. The assays of che-

motaxis and chemotactic inhibitory factor were done independently of each other by the two authors and the results collated retrospectively. A total of 13 pairs of assays in nine patients was performed. An HIE patient's blood was separated on Hypaque-Ficoll gradient, and the neutrophils' chemotaxis was assayed immediately while the mononuclear cells were placed into culture for 24 h before assay. A chemotactic defect was defined as being present when the *t* test comparing the average distance migrated by the control and HIE neutrophils had a *P* value of < 0.05. There was a significant concordance between the presence of an in vitro chemotactic defect and the production of an inhibitor of chemotaxis (*P* < 0.045 by Fisher's exact test).

**Specificity of production of an inhibitor of neutrophil chemotaxis by HIE mononuclear cells** The neutrophil chemotactic inhibitory activity of mononuclear cell supernatants obtained from normal males, females, and atopics as well as inhibitory activity from mononuclear cells of normal patients with bacterial infections, patients with chronic granulomatous disease (CGD), Chédiak-Higashi syndrome, hypogammaglobulinemia, and patients with increased IgE due to metazoan parasites was assessed (Table I).

There are no significant differences among normal males, females, or atopics. Although the IgE levels of all the atopics was not determined, two atopic patients with very high IgE levels comparable to those of HIE patients (9,000 and 33,000 IU/ml) showed no particular increase in inhibitor production (-9.8 and 9.6% inhibition, respectively). Mononuclear cell cultures from patients with high IgE (range, 220 to 5,600; mean, 2,810 IU/ml) due to metazoan parasites (*Schistosoma*, *Trichuris*, *Ascaris*) did not contain an inhibitory factor. Six determinations on four CGD patients

failed to reveal any significant production of chemotactic inhibitory factor (*P* > 0.20). Two patients with Chédiak-Higashi syndrome who were not in the accelerated phase of the disease produced moderate amounts of inhibitory factor. However, at no time did Chédiak-Higashi mononuclear cell cultures produce inhibition equal to the HIE supernatants that contained inhibitory activity (Fig. 2). When HIE mononuclear cells produced inhibition factor, a mean of 28.4±1.6% inhibition was seen as compared with 15.3±1.0% for Chédiak-Higashi patients (*P* < 0.01). Two hypogammaglobulinemic patients did not produce an inhibitory factor.

Thus, the presence of atopy, of infection, or of high IgE per se cannot explain the finding of increased chemotactic inhibitory activity in the supernatants of mononuclear cells of HIE patients. Three conditions that predispose to bacterial infections-CGD, Chédiak-Higashi syndrome, and hypogammaglobulinemia do not have the extent of inhibitory factor production seen in HIE.

**Production and characteristics of the chemotactic inhibitory factor** To see if the production of the inhibitory factor were time dependent, three HIE and seven normal mononuclear cell cultures were incubated for 72 h. As expected, the three HIE supernatants possessed chemotactic inhibitory activity at 72 h. However, five of seven normal supernatants produced significant inhibition when cultured for 72 h (3.6% at 24 h vs. 14.5% at 72 h, *P* < 0.01 paired sample *t* test). Therefore, given sufficient time, most normal mononuclear cells will produce a chemotactic inhibitory factor or factors.

Although 1:100 dilutions of mononuclear cell supernatants were used routinely in these studies, dose-response studies on two different supernatants revealed that significant inhibitory activity could be found at up to 1:2,000 dilution of 24-h HIE supernatants. A representative study is shown in Fig. 3.

To see if the factor was produced by lymphocytes, the mononuclear cell fraction for an HIE patient was twice exposed to a polystyrene plastic surface to deplete it of esterase-positive cells (monocytes). After the mononuclear cells were > 99% depleted of esterase-positive cells (0 of 200 cells counted were esterase positive), they were concentrated to 5 × 10<sup>6</sup>/ml and cultured as usual. Nondepleted mononuclear cells, supernatant diluted 1:100 produced 27.8% inhibition, while esterase-positive depleted cells produced 23.3% inhibition (*P* > 0.20). Cells adherent to polystyrene, which were comprised of ~50% esterase-negative cells, also produced inhibition. Because pure monocytes were not obtained, inhibitory factor production by monocytes could not be ruled out. However, the presence of esterase-positive cells (monocytes) was not

TABLE I  
Mean Percent Inhibition of Chemotaxis by Supernatants of Mononuclear Cells Diluted 1:100

	n*	Percent inhibition ±SEM
Normal males	7	7.2±4.3
Normal females	12	3.6±2.0
Atopic normals and patients	7	0.9±1.9
Infected patients	4	-4.7±2.7
Chronic granulomatous disease	6	2.2±5.8
Chédiak-Higashi syndrome	3	15.3±1.0
Hypogammaglobulinemia	3	1.4±4.0
Patients with parasites	5	7.3±2.4

\* n = number of determinations.

Subgroups of normal patients and other groups assayed for production of chemotactic inhibitory factor.

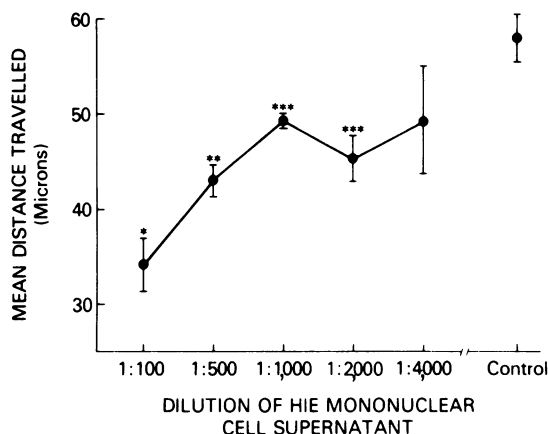


FIGURE 3 Dilutions of one HIE mononuclear cell supernatant assayed for chemotactic inhibitory factor (Note: abscissa is not linear). The control cells were exposed to only Hanks' solution. The chemoattractant used was 10 nM f-Met-Leu-Phe. \* $P < 0.001$  vs. control; \*\* $P < 0.01$  vs. control; \*\*\* $P < 0.05$  vs. control.

necessary for production of chemotactic inhibitory activity.

*Characterization and partial purification of the chemotactic inhibitory factor.* When inhibitory supernatants were heated to 56°C for 30 min, there was no loss of inhibitory activity. The inhibitory activity was destroyed when supernatants were treated with 10 mg/ml trypsin at 37°C or 10 mg/ml papain at 22°C. For the latter experiments the supernatants were treated for 30 min at 56°C to inactivate the enzymes before assay for inhibitory activity.

Preliminary gel filtration of HIE mononuclear cell supernatants indicated that the chemotactic inhibitory activity was in a pooled fraction of 20,000 to 80,000 mol wt. More detailed analysis on a G-100 column gave results typified by Fig. 4. Note that there is no detectable absorbance at 280 nm, indicating high biological activity of the material. Chromatography of three HIE supernatants yielded similar peaks of chemotactic activity. The mean molecular weight of the factor from these three determinations was 61,000. Normal mononuclear cell supernatants cultured for 24 h and HIE supernatants without detectable inhibitory activity did not reveal this peak. However, when normal mononuclear cells were cultured for 72 h, there was chemotactic inhibitory activity in the crude supernatant, and the 61,000-dalton inhibitory peak was detected.

Partially purified HIE chemotactic inhibitory factor from the G-100 column was exposed for 1 h in phosphate-buffered saline to two irreversible inhibitors of serine esterases, 1 mM PMSF, and 1 mM DFP. The material was then exposed to normal neutrophils, and

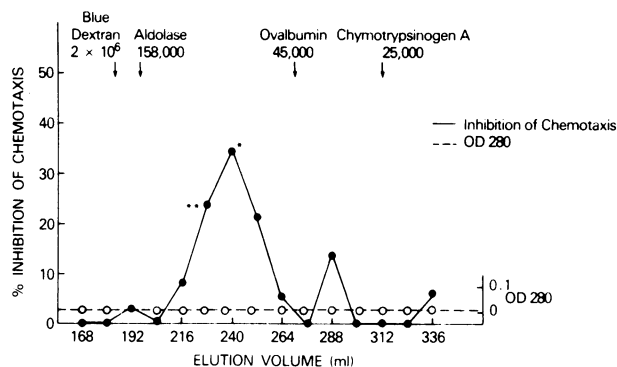


FIGURE 4 Chromatography of an HIE mononuclear cell supernatant on Sephadex G-100. Alternate tubes assayed for chemotactic inhibitory factor with tubes at elution volumes of 228 and 240 ml having significant inhibition of chemotaxis. OD<sub>280</sub> representing protein was negligible throughout the elution profile. \* $P < 0.01$ ; \*\* $P < 0.02$ .

a standard assay for chemotactic inhibition was performed. The activity of the PMSF and DFP was assayed by their ability to inhibit trypsin activity. Control experiments were done to assess the minimal chemotactic inhibition by the residual PMSF or DFP in the solutions. Table II is a summary of five such experiments. There was no significant decrease in inhibitory activity after treatment with either PMSF or DFP.

The chemotactic inhibitory factor is not a chemotactic factor that acts by causing chemotactic deactivation when PMN are exposed to it (15, 24). 10-

TABLE II  
Exposure of Partially Purified Chemotactic Inhibitory Factor to Serine Esterase Inhibitors

Inhibitor	Percent inhibition of chemotaxis		
	Experiment No.	Factor alone	Factor + PMSF*
PMSF, 1 mM	1	28.7	34.4
	2	22.0	16.0
	3	15.9	17.1
	mean±SEM	22.2±3.7	22.5±6.0§
DFP, 1 mM	4	14.4	14.3
	5	21.3	19.6
	mean±SEM	17.8±3.4	17.0±2.6§

\* 1 mM phenylmethylsulfonyl fluoride + chemotactic inhibitory factor at 37°C for 30 min.

† 1 mM diisopropyl fluorophosphate + chemotactic inhibitory factor at 37°C for 30 min.

§  $P > 0.20$  when compared with factor alone.

100-, and 1,000-fold dilutions of the partially purified inhibitory factor were not chemotactic (with only 100.5, 87.8, and 92.8% of the chemotactic activity of buffer alone). Furthermore, the partially purified inhibitory factor in concentrations sufficient to inhibit PMN chemotaxis did not cause release of lysozyme or lactic dehydrogenase from PMN, when compared with buffer-treated controls. Thus, inhibition of chemotaxis could not be a consequence of vigorous degranulation of the neutrophils exposed to the factor (21). Exposure of PMN to the chemotactic inhibitory factor also did not affect the ability of normal neutrophils to kill opsonized *Staphylococcus aureus* in an in vitro assay (percent bacterial survival at 90 min was 38% for normal neutrophils and 33% for neutrophils treated with partially purified inhibitory factor).

*Effect of the chemotactic inhibitory factor on f-Met-Leu-[<sup>3</sup>H]Phe binding.* In three separate experiments exposure of neutrophils to a concentration of partially purified inhibitory factor sufficient to inhibit chemotaxis did not affect the neutrophil's specific saturable binding of f-Met-Leu-[<sup>3</sup>H]Phe (factor-treated neutrophils had 96±6% of the specific binding of control cells). The displaceable bound f-Met-Leu-[<sup>3</sup>H]Phe was also not significantly changed by treatment with the factor (98±8% of control binding). The amount of nondisplaceable bound peptide was also not affected by the factor. Thus, it appears the decreased chemotactic response to f-Met-Leu-Phe cannot be explained by significant perturbations in f-Met-Leu-Phe receptor number or in internalization of bound f-Met-Leu-Phe.

*Effect of the chemotactic inhibitory factor on monocyte locomotion.* Testing of the crude supernatant for its ability to inhibit normal monocyte nondirected and chemotactic migration revealed that a 1:100 dilution of an HIE supernatant caused no decrease in monocyte nondirected migration (control = 192±28, factor treated = 157±36 cells/field). However, the crude supernatant decreased the number of monocytes that traversed the filter towards 10 nM f-Met-Leu-Phe chemoattractant (control = 403±17, factor treated = 300±27 cells/field,  $P < 0.02$ ). Treatment of mononuclear cells with a partially purified factor revealed similar results with no significant effect on nondirected migration (192±28 vs. 150±18 cells/field,  $P > 0.05$ ) and significant inhibition of chemotaxis (control = 411±48, factor treated = 259±45 cells/field,  $P < 0.05$ ). To rule out preferential inhibition of chemotaxis for monocytes or neutrophils, dose-response studies were performed with a range of HIE supernatant dilutions. Simultaneous assays of inhibition of monocyte and neutrophil chemotaxis revealed that there was significant inhibition of chemotaxis for dilutions of the HIE supernatant of 1/10, 1/50, 1/100, and 1/1,000 for both monocytes and neutrophils ( $P$

$< 0.05$  for each comparison of factor-treated cells with buffer-treated cells). Dilutions of 1/5,000 were not inhibitory ( $P > 0.20$ ) for either monocytes or neutrophils. Therefore, there is no preferential chemotactic inhibition by the HIE factor towards either monocytes or neutrophils as measured by an end-point dilution of inhibition assay.

## DISCUSSION

The HIE syndrome remains a series of enigmas, the foremost of which is the basic underlying defect or defects that cause its signs and symptoms. Two possible bases for recurrent infection include abnormal cell-mediated immunity and the presence of specific antistaphylococcal and anticandida IgE (7, 25). If abnormal cell-mediated immunity were the basis of recurrent infection in HIE, then we would expect infections with such bacteria as *Listeria*, *Nocardia*, and mycobacteria to be more common. However, the most common pathogenic organisms in HIE are *Staphylococci* and *Candida albicans*. There is good evidence that HIE patients have antistaphylococcal and anti-*Candida* IgE (7, 25), and it is possible that an infection with these organisms could lead to the combination of mast-cell bound IgE and staphylococcal antigens that might then lead to the release of mediators such as histamine that would alter leukocyte functions. Hill and Quie have suggested that histamine might play such a role in HIE (5), and there is evidence that H<sub>2</sub> antagonists improve neutrophil function in HIE in vitro (26) and in vivo (9). However, this mechanism cannot be the sole basis of recurrent infections in HIE as it will not explain the lack of similar recurrent infections in patients with anti-*Aspergillus* IgE in broncho-pulmonary aspergillosis or in the millions of people with parasites and antiparasite IgE or in patients with systemic mastocytosis.

We hypothesized that a defect in the immunoregulatory system of mononuclear cells might explain the features of this syndrome. To this end, we searched for an interactive mechanism between mononuclear cells and neutrophils that would be compatible with the clinical presentation of these patients. Since this work was begun, there have been two reports of deficient numbers of T cell suppressor cells in this syndrome (27, 28). This abnormality alone or in combination with other immunoregulatory defects could cause lymphocytes in the HIE syndrome to produce an inhibitor of neutrophil chemotaxis. This paper does not attempt to quantitate the numbers and percentage changes in suppressor or helper T cells with the production of a chemotactic inhibitory factor, although this may be the basis of the variation in production of the factor.



There is controversy in the literature regarding the existence of inhibitors of neutrophil chemotaxis in the serum of HIE patients (26, 29). We have not systematically studied our patients' serum for inhibitory factor although this should be a fruitful area for further study.

Several types of data argue for relevance of this inhibitor to the observed in vitro defects of HIE neutrophils: (a) its variable production in vitro seems to be correlated with the variable finding of an in vitro chemotactic defect, (b) the factor's interaction with neutrophils and monocytes affects their nondirected migration very little, if at all, while inhibiting chemotaxis to several chemotactic factors. The mean percentage inhibition of  $19.4 \pm 3.0\%$  may seem slight, but it correlates well with the percent inhibition seen in HIE neutrophils in vitro (mean of 10.5%). In addition, the factor does not damage the cells or cause them to degranulate their lysosomal enzymes. It does not impair neutrophils in the killing of staphylococci consistent with the observed normal killing by HIE neutrophils. It does not prevent binding of the chemotactic factor f-Met-Leu-Phe and, therefore, probably does not inactivate this chemotactic factor directly. The inhibitory factor is produced by normal mononuclear cells in 72-h culture and by HIE mononuclear cells in 24-h culture, indicating an abnormality in degree of production, not an absolute difference between normal and HIE mononuclear cells. Thus, the production of the factor as well as the factor's effect on neutrophils are both consistent with the in vitro findings one would expect if HIE patients' neutrophils were exposed to the factor in vivo. The only difference between the in vivo behavior of HIE patients' neutrophils and normal neutrophils treated with a 1:100 dilution of the HIE factor is in nondirected (random) motility. The small but significant decrease in HIE neutrophils' nondirected motility could not be correlated with the ability of the HIE inhibitory factor to alter normal neutrophil nondirected migration.

The identity of this chemotactic inhibitory factor is uncertain. It is clearly a cell-directed inhibitory factor, which is consistent with the finding of abnormal chemotaxis of HIE neutrophils in the absence of HIE serum or plasma. Its molecular weight is twelve times greater than the 5,000-dalton neutrophil-immobilizing factor described by Goetzl and Austen (30, 31). It has potent anti-PMN inhibitory activity unlike migratory inhibitory factor (32) and it has more than twice the molecular weight of migratory inhibitory factor. It likewise does not have the specificity or the mol wt of  $< 13,000$ , which is seen with the tumor-related macrophage chemotaxis inhibitor described by Snyderman et al. (33). A more likely identity of the HIE chemotactic inhibitor is to the leukocyte inhibitory factor

(LIF) described by Rocklin (32, 34, 35). LIF has a mol wt of 69,000 and has a preferential ability to inhibit neutrophil rather than monocytes chemotaxis. However, LIF is readily inactivated by exposure to the serine esterase inactivator DFP at 1 mM, whereas the HIE factor is not. Thus, the HIE chemotactic inhibitor seems to differ from LIF in its ability to inhibit monocyte chemotaxis as well as neutrophil chemotaxis and in its resistance to serine esterase inactivators.

The demonstration of a product from mononuclear cells of HIE patients and the correlation of the production of this product with a measurable in vitro chemotactic defect does more than suggest a possible basis for the recurrent infections in HIE patients. The finding of a mononuclear cell product, which may have direct relevance upon a defect in neutrophil function, opens new possibilities for further research in mononuclear cell-neutrophil interactions in more common clinical conditions such as chronic inflammatory diseases that have altered neutrophil function.

#### ACKNOWLEDGMENT

The authors wish to acknowledge the valuable statistical advice of Dr. David Alling.

#### REFERENCES

1. Davis, S. D., J. Schaller, and R. J. Wedgwood. 1966. Job's syndrome. Recurrent, "cold," staphylococcal abscesses. *Lancet*. I: 1013-1015.
2. Buckley, R. H., B. B. Wray, E. Z. Belmaker. 1972. Extreme hyperimmunoglobulinemia E and undue susceptibility to infection. *Pediatrics*. 49: 59-70.
3. Clark, R. A., R. K. Root, H. R. Kimball, and C. H. Kirkpatrick. 1973. Defective neutrophil chemotaxis and cellular immunity in a child with recurrent infections. *Ann. Intern. Med.* 78: 515-519.
4. Hill, H. R., and P. G. Quie. 1974. Raised serum-IgE levels and defective neutrophil chemotaxis in three children with eczema and recurrent bacterial infections. *Lancet*. I: 183-187.
5. Hill, H. R., H. D. Ochs, P. G. Quie, R. A. Clark, H. F. Pabst, S. J. Klebanoff, and R. J. Wedgwood. 1974. Defect in neutrophil granulocyte chemotaxis in Job's syndrome of recurrent "cold" staphylococcal abscesses. *Lancet*. II: 617-619.
6. Buckley, R. H., and W. G. Becker. 1978. Abnormalities in the regulation of human IgE synthesis. *Immunol. Rev.* 41: 288-314.
7. Schopfer, K. K., K. Baerlocher, P. Proce, U. Krech, P. G. Quie, and S. D. Douglas. 1979. Staphylococcal IgE antibodies, hyperimmunoglobulinemia E and *Staphylococcus aureus* infections. *New Engl. J. Med.* 300: 835-838.
8. Friedenbergl, W. R., J. J. Marx, Jr., R. L. Hansen, and R. C. Haselby. 1979. Hyperimmunoglobulin E syndrome: response to transfer factor and ascorbic acid therapy. *Clin. Immunol. Immunopathol.* 12: 132-142.
9. Mawhinney, H., M. Killen, W. A. Fleming, and A. D. Roy. 1980. The hyperimmunoglobulin E syndrome: a neutrophil chemotactic defect reversible by histamine

- H<sub>2</sub> receptor blockade? *Clin. Immunol. Immunopathol.* **17**: 483-491.
10. Wright, D. G., C. H. Kirkpatrick, and J. I. Gallin. 1977. Effects of levamisole on normal and abnormal leukocyte locomotion. *J. Clin. Invest.* **59**: 941-950.
  11. Gallin, J. I. 1980. Disorders of phagocyte chemotaxis. *Ann. Intern. Med.* **92**: 520-538.
  12. Gallin, J. I. 1975. Abnormal chemotaxis: cellular and humoral components. In *The Phagocytic Cell in Host Resistance*. J. A. Bellanti and D. H. Dayton, editors. Raven Press, New York. 227-248.
  13. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand. J. Clin. Lab. Invest.* **97**(Suppl.): 77-89.
  14. Gallin, J. I., R. A. Clark, and H. R. Kimball. 1973. Granulocyte chemotaxis: an improved in vitro assay employing <sup>51</sup>Cr-labeled granulocytes. *J. Immunol.* **110**: 233-240.
  15. Donabedian, H., and J. I. Gallin. 1981. Deactivation of human neutrophil chemotaxis by chemoattractants. Effect on receptors for the chemotactic factor f-Met-Leu-Phe. *J. Immunol.* **127**: 839-844.
  16. Zigmond, S. H., and J. G. Hirsch. 1973. Leukocyte locomotion and chemotaxis: new methods for evaluation and demonstration of a cell-derived chemotactic factor. *J. Exp. Med.* **137**: 387-410.
  17. Maderazo, E. G., and C. L. Woronick. 1978. A modified micropore filter. Assay of human granulocyte leukotaxis. In *Leukocyte Chemotaxis: Methods, Physiology and Clinical Implications*. J. I. Gallin and P. G. Quie, editors. Raven Press, New York. 43-55.
  18. Falls, W., R. H. Goodwin, Jr., and E. J. Leonard. 1980. A 48-well microchemotaxis assembly for rapid and accurate measurement of leukocyte migration. *Immunol. Methods.* **33**: 239-247.
  19. Hirsch, J. G., and B. Strauss. 1964. Studies on heat-labile opsonin in rabbit serum. *J. Immunol.* **92**: 145-154.
  20. Bergmeyer, H. U. 1963. *Methods of Enzymatic Analysis*. Academic Press, Inc., New York. 737-739.
  21. Gallin, J. I., D. G. Wright, and E. Schiffmann. 1978. Role of secretory events in modulating human neutrophil chemotaxis. *J. Clin. Invest.* **62**: 1364-1374.
  22. Dixon, W. J., and F. J. Massey, Jr. 1969. *Introduction to Statistical Analysis*. McGraw Hill Book Company, New York.
  23. Wilks, S. S. 1962. *Mathematical Statistics*. John Wiley and Sons, Inc., New York. 252.
  24. Ward, P. A., and E. L. Becker. 1968. The deactivation of rabbit neutrophils by chemotactic factor and nature of the activatable esterase. *J. Exp. Med.* **127**: 693-709.
  25. Berger, M., C. H. Kirkpatrick, P. K. Goldsmith, and J. I. Gallin. 1980. IgE antibodies to *Staphylococcus aureus* and *Candida albicans* in patients with the syndrome of hyperimmunoglobulin E and recurrent infections. *J. Immunol.* **125**: 2437-2443.
  26. Hill, H. R., R. D. Estensen, and N. A. Hogan. 1976. Severe staphylococcal disease associated with allergic manifestations, hyperimmunoglobulinemia E and defective neutrophil chemotaxis. *J. Lab. Clin. Med.* **88**: 796-806.
  27. Katona, I. M., G. Tata, R. T. Scanlon, J. A. Bellanti. 1980. Hyper IgE syndrome: a disease with suppressor T cell deficiency. *Ann. Allergy.* **45**: 295-300.
  28. Geha, R. S., E. Reinherz, D. Leung, K. T. McKee, Jr., S. Schlossman, and F. Rosen. 1981. Deficiency of suppressor T cells in the hyperimmunoglobulin E syndrome. *J. Clin. Invest.* **68**: 783-791.
  29. Matsumoto, T., Y. Gotoh, H. Narukami, Y. Miyamoto, and M. Honda. 1981. Case report: a hyperimmunoglobulin E syndrome with serum inhibitor against immune functions. *Ann. Allergy.* **46**: 86-88.
  30. Goetzl, E. J., and K. F. Austen. 1972. A neutrophil immobilizing factor derived from human leukocytes. I. Generation and partial characterization. *J. Exp. Med.* **136**: 1564-1580.
  31. Goetzl, E. J., and R. E. Rocklin. 1978. Amplification of the activity of human leukocyte inhibitory factor (LIF) by the generation of a low molecular weight inhibitor of PMN leukocyte chemotaxis. *J. Immunol.* **121**: 891-896.
  32. Rocklin, R. E. 1974. Products of activated lymphocytes: leukocyte inhibitory factor (LIF) distinct from migration inhibitory factor (MIF). *J. Immunol.* **112**: 1461-1466.
  33. Snyderman, R., M. C. Pike, and G. J. Cianciolo. 1980. An inhibitor of macrophage accumulation produced by neoplasms: its role in abrogating host resistance to cancer. In *Mononuclear Phagocytes. Functional Aspects*. R. van Furth, editor. Martinus Nijhoff Publishers, Hingham, MA. **1**: 569-590.
  34. Rocklin, R. E. 1975. Partial characterization of leukocyte inhibitory factor by concanavalin A-stimulated human lymphocytes (LIF<sub>con A</sub>). *J. Immunol.* **114**: 1161-1165.
  35. Rocklin, R. E., and A. S. Rosenthal. 1977. Evidence that human leukocyte inhibitory factor (LIF) is an esterase. *J. Immunol.* **119**: 249-252.