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PRIMARY INTRINSIC CHEMOTACTIC DEFECT*

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The prompt accumulation of neutrophils in tissues invaded by bacteria is essential for the suppression of bacterial growth and the prevention of possible infection. The experimental observations of Miles et al established that the absence of neutrophils from the tissue for even as short a time as 2 to 4 hours increases the infectivity of the inoculated bacteria as much as 100,000 fold[1]. The identification of functional abnormalities in the cellular and humoral components of leukocyte migration has contributed to our understanding of the mechanisms of impaired host defenses. In consequence, there has been a remarkable proliferation of reports on the clinical defects of these components in recent years[2-4].

The present report concerns a child with recurrent and widespread infections (periodontitis, sinusitis, chronic otitis media, bronchiectasis, giardiasis) and a primary intrinsic defect of neutrophil chemotaxis.

Case Report

An 11-year-old boy was referred to Hacettepe Children's Hospital in November 1979 because of a productive cough and otitis media since the age of 4 and recurrent episodes of lymphadenitis. Physical examination revealed an asthenic, chronically-ill looking child. His height and weight were below the third percentile. He had periodontitis and evidence of chronic tonsillitis, the left tympanic membrane was perforated and the cervical lymph nodes were enlarged, firm but not tender to palpation.

Examination of the chest revealed the presence of crepitant rales in both lung fields. He had hepatosplenomegaly 5 cm below the costal margins.

Laboratory examinations showed hemoglobin to be 11.25 gm/dl, blood leukocyte counts between 18,000 and 24,800 per mm^3, neutrophils 63-82%, lymphocytes

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18-28%, monocytes 3-5% and eosinophils 0-8%. X-ray examination revealed bilateral maxillary sinusitis, chronic mastoiditis and bronchiectasis. Sweat chloride was 21 mEq/l. Bone marrow aspiration failed to show any pathologic findings. Stool examination for ova and parasites showed the presence of Giardia lamblia. A liver needle biopsy showed nonspecific hepatitis.

Serum immunoglobulin levels (IgG 1150, IgM 200, IgA 171 mg/dl) were within normal limits. Serum anti-B titer was 1/512, skin tests for delayed hypersensitivity to PHA and SKSD (streptokinase-streptodornase) were positive, while skin tests for candida and PPD were negative. E 65%, EA 14% and EAC 11% rosette test results were normal. Neutrophil EA 65% and EAC 84% rosettes were also within normal limits. Lymphocyte blastogenic transformation to PHA was normal.

Histochemical staining for neutrophils and monocytes demonstrated normal peroxidase activity. Leukocyte alkaline phosphatase ($86.2/10^8$ leukocytes), total complement hemolytic activity (CH_{50}:1/23), NBT reduction and glucose-6 phosphate dehydrogenase (G_6PD) spot tests were also found to be normal.

Material and Methods

Leukocyte fractions: Polymorphonuclear leukocytes (PMN) and rich fractions of peripheral blood leukocytes were obtained by the two-step technique of Boyum [5] and neutrophil counts adjusted to 3×10^7/ml. Viability after the procedures was greater than 93 percent.

Chemoattractants: Zymosan-activated plasma (ZAP) (5 mg zymosan-A (sigma)) was added to 1 ml of plasma, incubated 45 minutes at 37°C and then centrifuged at 2000 rpm for 10 minutes. Supernatant was used. E. coli culture filtrate and casein 5 mg/dl (sodium caseinate-Difco) were also used as chemoattractants.

Chemotaxis under agarose was performed with some modifications by the method of Nelson et al[6,7].

The membrane filter method was performed as described by Wilkinson[8]: 2×10^6 PMN were added to the upper side and 20% E. coli culture filtrate was added to the lower side. After a 3-hour incubation, the cells which traveled across the 3-micron filter were counted at 5 different high power fields. Every experiment was done in triplicate with both the agarose and the filter method.

Immunoglobulins were determined by radial immunodiffusion. E, EA, EAC rosettes and lymphocyte blastogenic transformation were determined as previously described [9,10].

Results

The patient's cellular and humoral immunity tests were all within normal limits (Table I).

TABLE I

RESULTS OF CELLULAR AND HUMORAL IMMUNITY TESTS

	Skin tests				Total lymphocytes	Immunoglobulins (mg/dl)			Anti-B	Rosettes		
	PHA	Cand.	SKSD	PPD		IgG	IgA	IgM		E%	EA%	EAC%
Patient	6 × 10 mm	(–)	5 × 5 mm	(–)	4320/mm^3	1150	171	200	1/512	65	14	11
Controls					> 2000/mm^3	1190 ± 370	232 ± 58	114 ± 39		69 ± 8	12 ± 5	17 ± 6.7

Chemotoxigenicity of the patient's plasma and serum after activation with zymosan was normal and did not show inhibitor activity when mixed with normal plasma. The chemotactic activity of the patient's PMN was tested on seven separate occasions within a six-month period. Each time, chemotactic activity was found to be considerably lower than the mean of 20 normal adults and 11 normal age-matched (5-13 years) controls (Table II).

TABLE II

NEUTROPHIL CHEMOTAXIS

	Zymosan-activated plasma (ZAP)	E. coli	Casein	Medium	
Agarose method					
Patient's PMN*	350 ± 64	366 ± 79	333 ± 86	264 ± 68	(μm)
	211 ± 57	165 ± 71	196 ± 79	138 ± 51	(cells)
20 adult controls' PMN	869 ± 194	831 ± 169	806 ± 197	460 ± 115	(μm)
	894 ± 348	799 ± 336	729 ± 373	373 ± 172	(cells)
11 age-matched controls' PMN	874 ± 220	841 ± 269	881 ± 170	493 ± 123	(μm)
	950 ± 269	841 ± 377	800 ± 377	457 ± 175	(cells)
Filter technique					
Patient's PMN	not done	28 ± 4	not done	19 ± 3	(cells)
Controls' PMN	not done	126 ± 16	not done	38 ± 6	(cells)

* Mean ± standard deviation of seven experiments

Incubation of normal PMN in the patient's plasma or serum for 1 hour at 37°C did not inhibit the chemotaxis. Furthermore, normal serum or plasma failed to correct the chemotactic activity of the patient's PMN.

Efforts were made to obtain normal chemotactic response by prolonging the incubation time and increasing the cell count. Although migrating cell counts and distance increased with these parameters, they never reached the normal values (Tables III-IV).

Discussion

Patients with disorders of granulocytic origin are subject to frequent infections. Their clinical manifestations are often quite similar regardless of whether the disorder is due to insufficient numbers of cells or cell dysfunction. Infections tend to be prolonged and recurrent, and there is poor response to appropriate antibiotics.

TABLE III

EFFECT OF INCREASING CELL NUMBERS ON NEUTROPHIL CHEMOTAXIS*

Cell count	Patient's PMN		Controls' PMN	
1.5×10^5	125 μm	120 cells	800 μm	760 cells
2×10^5	200 μm	110 cells	900 μm	815 cells
3×10^5	250 μm	173 cells	1050 μm	1195 cells
4×10^5	300 μm	186 cells	1100 μm	1248 cells
6×10^5	425 μm	313 cells	1250 μm	1443 cells

* E. coli culture filtrate was used in these experiments.

TABLE IV

EFFECT OF INCUBATION TIME ON CHEMOTAXIS

Incubation time	Patient's PMN		Controls' PMN	
2 hr	150 μm	110 cells	700 μm	721 cells
3 hr	275 μm	135 cells	1050 μm	963 cells
4 hr	325 μm	169 cells	1250 μm	1145 cells
5 hr	375 μm	198 cells	1350 μm	1251 cells

Infections most typically involve soft tissues including skin, lungs, liver and kidneys. Pneumonia occurs frequently. Deep tissue abscesses also occur, but systemic bacterial disease is uncommon [2-8].

Our patient's PMN demonstrated abnormalities in vitro which were manifested by significantly decreased chemotaxis and random migration. Cellular and humoral immunity tests were normal. In vitro studies with normal PMN in the presence of the patient's serum and plasma demonstrated that the chemotactic abnormalities were cellular in nature rather than secondary to serum factors or an inability to generate chemotactic substances. The locomotion defect in our patient was characterized by the abnormally directed, random migration seen in lazy leukocyte syndrome[11-12]. In contrast to our patient, however, three patients reported with this syndrome had severe neutropenia and failed to respond to infections with neutrophilia. During the 6-month period, our patient never had neutropenia and responded to active infection normally with neutrophilia as high as 20,300 PMN/mm^3.

Patients with Chediak-Higashi syndrome have been demonstrated to possess abnormal chemotaxis, normal random migration and decreased bactericidal ability[13,14]. These patients are clinically easy to distinguish from cases such as ours by their partial oculo-cutaneous albinism and characteristic giant lysosomal inclusions in the leukocytes.

Patients with Job's syndrome[15], atopic dermatitis[16], mannosidosis[17], Down's syndrome[18], hyper IgE syndrome[19], diabetes mellitus[20], congenital ichthyosis[21], and severe burns[22] have been reported to have defective chemotaxis but normal random migration. The clinical and laboratory findings of our case do not match these entities. Defective chemotaxis and random locomotion were reported in some isolated cases with obvious morphologic and enzymatic abnormalities of neutrophils which we could not detect[23,24]. Boxer et al reported a patient with recurrent staphylococcal infections who had a markedly abnormal cutaneous inflammatory response and in vitro PMN chemotactic abnormalities[25]. This individual also displayed abnormal random locomotion and decreased phagocytic rate. A structural abnormality attributed to an abnormal leukocyte actin-like protein was also noted. These findings have some similarity to ours. Related studies on underlying causes of this chemotactic defect and the phagocytic and bactericidal activity of neutrophils are currently under investigation. Although the relation between this chemotactic defect and the patient's increased susceptibility to infection has been fully elucidated, the continued study of such patients is necessary for delineating and understanding the role of chemotaxis in immunologic function.

Summary

An 11-year-old boy with a history of recurrent and widespread pyogenic infection had abnormal neutrophil chemotaxis which probably accounted for his susceptibility to infection. Cellular and humoral immunity tests were normal.

This patient represents another of the growing number of cases with distinct chemotaxis defects, currently being recognized as increasing susceptibility to infection.

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