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INTRINSIC DEFECT OF THE POLYMORPHONUCLEAR LEUCOCYTE RESULTING IN IMPAIRED CHEMOTAXIS AND PHAGOCYTOSIS

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SUMMARY

A patient is presented who has recurrent infections associated with a cellular defect of PMN chemotaxis and phagocytosis, as well as a sex-linked form of congenital agammaglobulinaemia. The impairments of PMN function were demonstrated *in vitro* by an inability of the patient's PMNs to respond to chemotactic factors, by an inability of the PMNs to phagocytize *S. aureus*, and by abnormal NBT tests. These abnormalities were not corrected by the addition of normal serum or plasma to the patient's PMNs. No evidence was obtained for a plasma inhibitor of PMN function.

INTRODUCTION

The availability of suitable techniques for the *in vitro* measurement of polymorphonuclear leucocyte (PMN) function has made it possible to identify and characterize a number of clinically significant defects of chemotaxis and phagocytosis (Alper *et al.*, 1970; Miller & Nilsson, 1970; Ward & Schlegel, 1969). In each case, the chemotactic or phagocytic defect was a consequence of an abnormality in humoral, not cellular, function. In this report, we describe for the first time a patient with recurrent infections associated with a unique cellular defect of PMN chemotaxis and phagocytosis, as well as a sex-linked form of congenital agammaglobulinaemia.

CASE REPORT

J. G. is a 3½-year-old white male who presented with left upper and lower lobe pneumonia.

Past medical history. The patient was the product of a term pregnancy, prolonged labour, and normal delivery; birth weight was 6lb 10 oz. He was well until 3 months of age when he developed recurrent respiratory infections associated with high fevers, which were treated

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successfully with antibiotics. Since the age of 18 months, superficial abscesses, delayed healing and recurrent infection from minor cuts and insect bites have been noted. At 2 years of age, he had the first of nine episodes of pneumonia, all of which were documented by chest X-rays. He recovered uneventfully from chicken pox and tolerated immunization with oral polio vaccine and DPT.

Family history. The patient has two maternal male first cousins with documented agammaglobulinaemia who have not responded to conventional γ-globulin therapy. In addition, a maternal uncle and great-uncle had histories of recurrent infections and died in childhood. The patient's only sibling died of intestinal obstruction in the newborn period.

Clinical course. On admission to the hospital, the patient was febrile and tachypneic. He had nasal flaring, intercostal retractions, and grunting respirations. Rales were present over the left anterior and posterior lung fields; dullness to percussion and markedly decreased breath sounds were present in the left lower lung field. There was no hepatosplenomegaly. Cervical and axillary nodes were palpable. Numerous indurated crusted skin lesions were present on the extremities. After cultures were obtained, he was treated with ampicillin and kanamycin. He gradually improved clinically over a 2-week period. Following discharge from the hospital and even after γ-globulin therapy was instituted, a residual indolent pneumonia and skin infections have persisted.

Laboratory findings. The patient had a haemoglobin of 10·4 g% and a haematocrit of 30%; his WBC was 13,300 with 62% mature PMNs, 31% lymphocytes, and 7% monocytes. Chest X-ray revealed left lower and upper lobe infiltrates. Sweat chloride was normal. Quantitative immunoglobulin determinations revealed: IgG 34 mg%, IgM undetectable, IgA 8·7 mg%, IgD 2·7 mg%, and IgE 0·021 mg% (IgG, IgM, and IgA levels, markedly decreased: IgD and IgE levels, normal)*. The patient had no febrile agglutinins, no anti-A iso-haemagglutinin (his blood group was B), and a positive Shick test. He had no antibody response to immunization with typhoid antigen. Normal lymphocyte transformation was demonstrated with phytohaemagglutinin, pokeweed mitogen, and diphtheria antigen. A *Candida albicans* skin test was positive at 48 hr.

MATERIALS AND METHODS

Leucocyte suspensions were obtained from freshly drawn heparinized blood after sedimentation in 6% dextran solution for approximately 20 min. For studies of chemotaxis, the erythrocytes contaminating the buffy coat preparations were removed by hypotonic lysis with 0·2% NaCl for 20 sec followed by the addition of an equal volume of 1·6% NaCl to restore isotonicity. The leucocytes were then washed and resuspended in Gey's balanced salt solution (Snyderman, Gewurz & Mergenhagen, 1968) to a final concentration of $2{\cdot}2 \times 10^6$ PMNs/ml. Phagocytosis studies were performed on leucocyte suspensions which had been washed twice in Hanks's buffered salt solution (HBSS) and resuspended in a volume of HBSS to yield a concentration of 1×10^7 PMNs/ml.

In vitro chemotaxis was estimated using a modification of Boyden's technique as described previously (Snyderman *et al.*, 1969; Temple *et al.*, 1970). In these experiments, a 5·0 u filter was used in the chemotactic chamber. In most of the experiments, the chemotactic

* IgG, IgM, IgA, and IgD were determined through the courtesy of Dr William Terry at the Immunoglobulin Reference Center (Bethesda, Maryland). IgE determination was kindly performed by Dr Stephen Polmer, NIH.

factor placed in the lower chamber was generated by incubating 0·2 ml of normal human serum with 200 μg of endotoxin (*Salmonella typhosa* 0901, Difco). Partially purified preparations of C5a were obtained by incubating endotoxin with normal serum and then isolating the active C5a fragment by Sephadex G-100 chromatography (Snyderman & Mergenhagen, 1970). The Rebuck skin window technique for the detection of *in vivo* chemotaxis was performed as described by Rebuck & Crowley (1955).

Histochemical nitroblue tetrazolium (NBT) dye tests were performed as described by Windhorst *et al.* (1967). Quantitative NBT tests were performed as described by Baehner & Nathan (1968).

Bactericidal activity of the PMNs was estimated by the method of Alexander, Windhorst & Good (1968). Mixtures consisting of 5×10^6 PMNs, 5×10^6 test organisms in HBSS containing 1·0% gelatin, and normal serum or plasma were agitated gently at 37°C for a period of 4 hr. The PMNs were then lysed in distilled water and the number of viable bacteria were estimated with a surface colony counting technique. This test measures viable extracellular, as well a intracellular, bacteria. The viable intracellular bacteria were quantitated by incubating the PMN-bacterial suspensions in the presence of antibiotics (penicillin 100 u/ml, and streptomycin 100 μg/ml) which kill the extracelluar bacteria. At the appropriate times, the suspensions were centrifuged, washed, lysed in distilled water, and plated. With these procedures, it is possible to determine whether a bactericidal defect is due to a phagocytic or intracellular killing defect.

For complement titrations, blood was drawn and allowed to clot at room temperature for approximately 30 min. The serum was then removed and stored at −40°C for less than 2 weeks prior to use. The reagents and methods for titrations of whole complement, C1, C4, and C2 were described by Rapp & Borsos (1970). The haemolytic activities of C3 and C5 were determined by a modification of the procedures given in reference (Shin & Mayer, 1968).

A biopsy of a skin lesion was obtained, fixed, sectioned and stained with haematoxylin and eosin (H&E) and Gram stains.

RESULTS

Chemotaxis. Experiments were performed to test the ability of the patient's PMNs to respond to factors known to be chemotactic *in vitro* (Snyderman & Mergenhagen, 1970) and to test the ability of the patient's serum to generate chemotactic activity upon interaction with bacterial endotoxin. In addition, the patient's serum was studied to determine whether it contained an inhibitor of PMN chemotoactic activity. The results of these experiments are presented in Table 1. It is seen that the patient's PMNs failed to respond normally either to chemotactic factor(s) generated by the interaction of normal serum with endotoxin or to a preparation of partially purified C5a. Moreover, the patient's serum, upon interaction with endotoxin, was relatively deficient in its capacity to generate chemotactic activity for normal PMNs. There was no evidence that the poor response of the patient's PMNs to chemotactic factor(s) was a consequence of an inhibitor present in his serum since normal PMNs were capable of a normal chemotactic response in the presence of the patient's serum. In addition, incubation of the patient's PMNs with normal serum did not correct the defect. The PMNs obtained from the patient's parents were tested for their ability to respond chemotactically to partially purified preparations of C5a. The response of their PMNs was not significantly different from that of normal controls.

NBT dye studies. The results of the histochemical NBT test given in Table 2 show both a decreased ability of the patient's PMNs to ingest latex particles and a decreased ability of those cells which had ingested the particles to reduce the dye. These defects were not corrected by incubating the patient's cells in normal plasma. Furthermore, there was no evidence that the patient's plasma inhibited either the uptake of particles or reduction of the dye by normal PMNs. Quantitative estimation of the ability of the patient's cells to reduce NBT dye revealed a change in optical density of 0·037 (normal, 0·130–0·370). The patient's mother and father each had normal quantitative and histochemical NBT tests.

TABLE 1. Chemotaxis*

PMNs	PMNs suspended in medium containing	Source of chemotactic factor		
		Patient's Serum	Control Serum	C5a
P		120 PMNs/HPF	252 PMNs/HPF	38 PMNs/HPF
C		360	600	354
P	patient serum†	26	175	—
P	control serum†	25	140	—
C	patient serum†	264	590	—
C	control serum†	192	300	—

* The results expressed are representative of a number of similar experiments.
† Final concentration—10%.
P—Patient.
C—Control.

Bactericidal activity. The results summarized in Table 3(A) show that when the patient's PMSs were incubated with *Staphylococcus aureus* in the absence of antibiotics, approximately 70 times more residual viable bacteria remained than when control PMNs were used. This indicated that the patient had a defect in either phagocytosis or intracellular killing. Incubation of the patient's cells with bacteria in the presence of antibiotics showed approximately the same number of bacteria remaining as when control PMNs were used, suggesting that the bactericidal defect was primarily a consequence of impaired phagocytosis. Furthermore, there was no significant correction of this defect in the presence of normal

TABLE 2. Histochemical NBT dye studies

PMNs	Plasma	PMNs with ≥ 1 latex particles × 100	PMNs with reduced dye × 100
		Total number of PMNs	PMNs with > 9 latex particles
P	P	50	49
P	C	56	50
C	P	85	82
C	C	82	85
N	N	75–99	75–99

P—Patient; C—Control; N—Normal children.

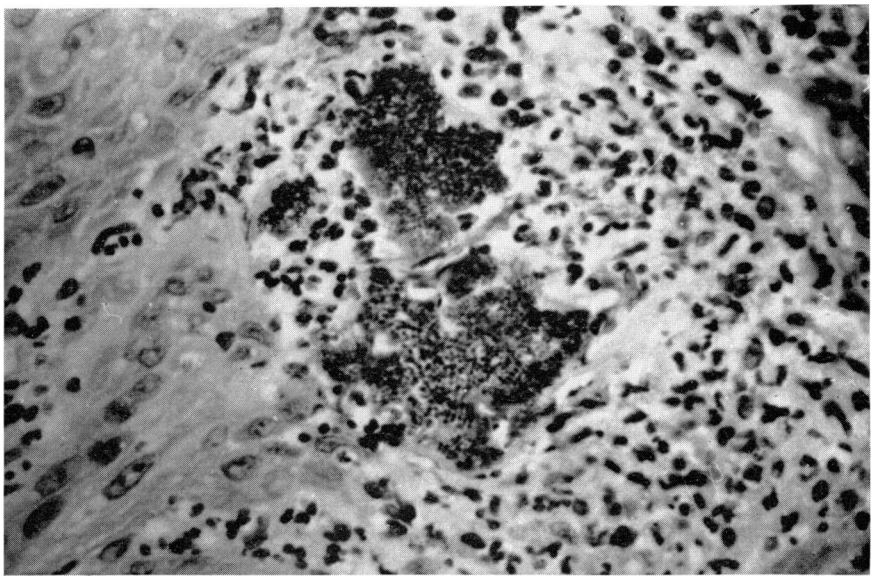

FIG. 1. Histologic appearance of skin lesion. Lesion consisted of sub-epidermal collections of Gram-positive cocci in clumps surrounded by reduced numbers of PMNs showing no evidence of intracellular bacteria, vacuolization, or lysis. A mononuclear infiltrate (without plasma cells) surrounds the PMNs (H & E, × 720).

plasma. Normal PMNs were capable of normal phagocytosis even in the presence of patient's plasma. In contrast, the results shown in Table 3(B) indicate that the patient's PMNs were capable of normal phagocytosis and intracellular killing of *Escherichia coli*. The parents' PMNs had normal bactericidal activity for *S. aureus*.

Histology. The paucity of PMNs in sections of a biopsy of an indurated skin lesion and the

TABLE 3. Bactericidal activity

Test organism	PMNs	Plasma	Percentage of viable bacteria remaining at 4 hr	
			Without antibiotics	With antibiotics
(A) *S. aureus*	P	P	49	0·005
	P	C	37	0·003
	C	P	0·88	0·001
	C	C	0·39	0·002
	N	N	<4	<0·01
(B) *E. coli*	P	P	0·15	—
	P	C	0·74	—
	C	P	0·20	—
	C	C	0·54	—
	N	N	<4	—

P—Patient; C—Control; N—Normal subjects.

failure to detect intracellular bacteria, vacuolization, and lysis in those PMNs in the field (Fig. 1) provided evidence for *in vivo* defects in chemotaxis and phagocytosis. In addition, the chemotactic defect was also demonstrated *in vivo* by a failure to detect a PMN exudate on challenge of the patient with the Rebuck skin window test.

Serum complement. The results given in Table 4 show that the patient's serum contained normal to slightly elevated concentrations of haemolytically active whole complement, C1, C2, C3, C4, and C5.

DISCUSSION

Normal chemotaxis and phagocytosis are a consequence of opsonic and chemotactic factors derived from serum interacting with cells capable of responding to these factors. In this report, we describe the first example of an impairment of chemotaxis and phagocytosis which is primarily the result of an intrinsic defect of polymorphonuclear leucocytes. These impairments of PMN function were demonstrated by an inability of the patient's PMNs to respond to chemotactic factors in 'activated' whole serum or in preparations of C5a, by an

TABLE 4. Complement concentrations in serum

Serum	Haemolytic titres*					
	Total C	C1	C2	C3	C4	C5
Patient	50·1	50,600	4,790	3,620	144,000	6,400
Normal controls	35–45	50–150,000	2–4,000	2,500	50–150,000	3,000

* Total C activity in CH_{50} units.
Individual Components C1–C5 expressed as the dilution of serum yielding 63% lysis (an average of one effective molecule per indicator cell).

inability of PMNs to phagocytize *Staphylococcus aureus in vitro*, and by abnormal PMN histochemical and quantitative NBT tests. Since these abnormalities were not corrected by the addition of normal serum or plasma to the patient's cells, the deficiencies in chemotaxis and phagocytosis could not be accounted for solely by a deficiency of a humoral factor. Moreover, no evidence was obtained for the presence of a humoral inhibitor of PMN function, since the patient's serum and plasma failed to inhibit the chemotactic and phagocytic capacities of normal PMNs. Although this patient is agammaglobulinaemic, it is clear that his *in vitro* defect of chemotaxis and phagocytosis is not primarily a consequence of reduced antibody levels since normal serum or plasma, which would provide such antibodies, failed to completely correct these defects. It should be noted, however, that the patient's phagocytic defect is a selective one since his PMNs were capable of normal phagocytosis and intracellular killing of *E. coli*.

With the exception of chronic granulomatous disease (CGD), the previously reported abnormalities of granulocyte function have been attributed to abnormalities of the serum. These abnormalities are identified either by the ability of normal serum or serum factors to correct the dysfunction or by the demonstration of a serum inhibitor of PMN function (Alper *et al.*, 1970; Miller & Nilsson, 1970; Ward & Schlegel, 1969; Mickenberg, Root &

Wolff, 1970; Miller *et al.*, 1968). In this patient, neither a deficiency of a humoral factor nor an inhibitor of PMN function was identified. Chemotactic and phagocytic defects have been reported in association with abnormalities of the serum complement system (Alper *et al.*, 1970; Miller & Nilsson, 1970). Since this patient had normal or elevated levels of haemolytic activity for total complement and the individual components of C1 through C5 and failed to show correction of PMN function with the addition of normal serum, it is improbable that an abnormality of the complement system is responsible for his defects in PMN function. In addition to an intrinsic PMN dysfunction, his serum was deficient in its ability to generate chemotactic factor(s) when his serum was interacted with bacterial endotoxin. This was possibly due to a lack of 'natural' antibody to endotoxin.

The major defect in CGD is manifested by an inability to kill certain bacteria intracellularly but not by an inability to ingest the organisms. The cellular defect in CGD can be detected by an impaired bactericidal activity of the PMNs for *Staphylococcus aureus*, *Serratia marcescens*, and other Gram-negative bacteria, and by an abnormal NBT dye test which depends on the hexose monophosphate shunt activity (Good *et al.*, 1968). The patient described in this report had impaired bactericidal activity for *S. aureus;* however, this defect was mainly due to impaired phagocytosis, whereas the patients with CGD have impaired intracellular killing. In his case, the decreased ability to reduce NBT dye was primarily due to a decreased ability of his PMNs to phagocytize the latex particles. However, of those granulocytes capable of phagocytizing the latex particles, a smaller proportion than normal were capable of reducing the dye. This finding suggests that, in addition to a chemotactic and phagocytic defect, the patient's cells may also be deficient in other PMN functions related to the hexose monophosphate shunt activity.

At the present time, it is not certain whether these intrinsic granulocytic dysfunctions are the result of an acquired or inherited defect. Nevertheless, detailed studies of the structural and biochemical characteristics of this patient's PMNs should provide an opportunity to define the metabolic and morphologic basis of chemotactic and phagocytic functions.

Recently, two additional patients have been reported with intrinsic granulocytic defects (Higgins, Swanson & Yamagaki, 1970; Miller, Oski & Harris, 1970). The granulocytes obtained from these patients demonstrated an abnormality of cell migration (i.e. chemotaxis) in the presence of normal phagocytic function.

These reports, as well as the patient described here, suggest that a spectrum of intrinsic granulocytic defects may be responsible for impaired granulocytic function.

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