Impaired neutrophil function in intestinal lymphangiectasia

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SUMMARY Impaired neutrophil chemotaxis and phagocytosis were shown in three patients with intestinal lymphangiectasia. Abnormalities in cell associated and serum derived activity occurred and possible mechanisms are suggested.

Intestinal lymphangiectasia is a disease characterised by dilated lymphatics, protein losing enteropathy, hypoalbuminaemia, and oedema; and patients with this disease lose albumin, immunoglobulins, and lymphocytes into the bowel.^{1 2} The condition may be primary, or secondary to underlying diseases—such as Crohn's disease, Whipple's disease, Behcet's syndrome, and cardiac failure.³ Patchy lymphatic dilatation is seen on small bowel biopsy specimens, and radiology may show characteristically oedematous mucosal folds with tiny filling defects caused by congested lacteals. The limb lymphatics are also commonly affected in primary intestinal lymphangiectasia, and lymphoedema may be severe.

Patients with intestinal lymphangiectasia have impaired cell mediated immunity, on skin testing and in vitro.¹ This has been attributed to continuous loss of lymphocytes into the bowel lumen,⁴ but despite lymphopenia and hypogammaglobulinaemia, the consequences of impaired cellular immunity seem to be relatively few. Opportunistic infection is unusual,² although severe cutaneous viral infections may occur.^{5 6} These patients, however, may show prolonged poor health with recurrent, though relatively minor, bacterial infections of skin, respiratory and urinary tract, despite a generally satisfactory overall nutritional state achieved by dietary supplementation.^{7 8}

Neutrophils have a major role in the body's defence against bacterial infection, but we are not aware of any previous reports of granulocyte function in intestinal lymphangiectasia. We report three patients with primary intestinal lymphangiectasia in whom standard in vitro tests of neutrophil function were abnormal.

Patients and methods

The Table shows patient details. All three patients had a documented protein losing enteropathy and histological confirmation of lymphangiectasia on small bowel biopsy. Secondary disease was excluded by appropriate investigations. Two patients (cases 1 and 2) showed hypoplastic lower limb lymphatics on lymphangiography. Case 1 had had severe disease throughout childhood and had gross lymphoedema. He experienced recurrent cutaneous and respiratory tract infections and had extensive cutaneous viral warts. Case 2 also had severe disease with oedema and recurrent pleural effusions and, in addition, severe peripheral vascular disease.

All three patients were stable at the time of investigation, with no evidence of current bacterial infection: they were receiving treatment with a low fat diet and dietary supplements, including vitamins and medium chain triglycerides. Complement (C3 and C4) values were normal in all patients, and none had evidence of spontaneous complement activation.

ASSESSMENT OF NEUTROPHIL FUNCTION

Neutrophils from patients and healthy controls were separated from fresh heparinised blood by centrifugation on a Ficoll-metrizoate (Lymphoprep, Pharmacia) density gradient and sedimentation in 3% dextran. Contaminating erythrocytes were lysed by the addition of 0.87% Tris buffered ammonium chloride. The remaining pellet of neutrophils was washed in medium (RPMI, Gibco) and resuspended at a final concentration of 2×10^6 /ml. Isolated neutrophils were 95% pure and 98% viable, as based on trypan blue exclusion.

CHEMOTAXIS

This was measured using a modification of the

Table Clinical and laboratory details of three patients with intestinal lymphangiectasia

Case No	Sex	Age (years)	Age at diagnosis (years)	Albumin (g/l) (n > 36)	Total immunoglobulin (g/l) (n > 9.5)	Lymphocyte count $\times 10^9/l$ (n > 1.5)	Polymorphonuclear count $\times 10^9/l$ (n > 2.5)	Fibronectin (mg/ml) (n > 200 mg/l)
1	M	17	3	23	4.7	0.4	2.7	125
2	M	49	40	28	8.6	1.1	7.9	110
3	F	67	66	20	5.0	1.4	4.2	145

Boyden method.⁹ Serum activated by zymosan was used as the chemoattractant, and the distance migrated through the millipore filter (pore size 3 μ m) was determined using the leading front method. Each test was performed in triplicate.

PHAGOCYTOSIS

This was assessed by incubating neutrophils with heat killed *Candida albicans* for 30 minutes at 37°C. Each assay was performed in duplicate and after incubation the percentage of 200 cells ingesting *Candida* was counted on a smear slide stained with Giemsa. Neutrophil phagocytosis was taken as the mean percentage of cells ingesting *Candida*.

The normal ranges for chemotaxis and phagocytosis were taken as the mean (2SD) of the values previously obtained in 40 healthy volunteers.

FIBRONECTIN

This was measured in citrated plasma by radial immunodiffusion (Serotec).

Analysis was performed using the Mann-Whitney U test.

Results

Neutrophil morphology in the patients was normal with no excess of non-segmented, immature, or toxic forms.

CHEMOTAXIS

Random unstimulated neutrophil migration in the absence of serum was similar 22 (5) μ m in both patients and controls.

Neutrophils from the patients with intestinal lymphangiectasia showed significantly reduced chemotaxis towards autologous 45 (12) μ m and normal 42 (23) μ m activated serum compared with that of control cells ($p < 0.05$) (Fig. 1). Normal neutrophils showed impaired chemotaxis towards patients'

Fig. 1 Chemotaxis by neutrophils from patients with lymphangiectasia and from controls towards zymosan activated serum (patient and control).

Fig. 2 Phagocytosis of heat killed *Candida albicans* by neutrophils from patients with lymphangiectasia and from controls in presence of patient and control serum.

activated serum 57 (11) μ m compared with that of normal serum 78 (2) μ m ($p < 0.05$) (Fig. 1).

PHAGOCYTOSIS

Phagocytosis by neutrophils from the patients with intestinal lymphangiectasia was impaired in the presence of autologous 37 (5)% and normal serum 37.5 (5)% compared with that of control cells ($p < 0.05$) (Fig. 2). Phagocytosis by normal neutrophils was impaired in the presence of patients' sera 40 (3)% compared with that of normal serum 52 (4)% ($p < 0.05$) (Fig. 2).

FIBRONECTIN

These values were subnormal in all patients.

Discussion

The present study indicates that the neutrophil functions of chemotaxis and phagocytosis may be impaired in patients with intestinal lymphangiectasia, in terms of cellular and serum values. Reduced chemotaxis by patients' cells in the presence of normal serum indicates a defect within the cell, preventing normal migration (Fig. 1). Chemotaxis is a complex process requiring recognition of a chemoattractant, orientation within its chemical gradient, and directed migration along that gradient.¹⁰ Specific receptors for various chemoattractants, including activated complement,¹¹ bacterially derived peptides,¹² and fibronectin¹³ have all been identified on the neutrophil membrane, and a change in receptor affinity, number, availability, or type might account for the present observations. Alternatively, the defect may lie further along the sequence of events required for normal chemotaxis; and abnormalities in microtubule formation¹⁴ and cellular adherence¹⁵ have occasionally been described in other conditions, leading to impaired migration.

Abnormal neutrophil mobility may occur in association with poor nutritional states in infants¹⁶ and children¹⁷ but, although our patients were hypoalbuminaemic, their overall nutrition was satisfactory. Both impaired¹⁸ and enhanced¹⁹ mobility have been described in patients with active bacterial infection, but our patients had no evidence of current infection at the time of study. It has been suggested that immature neutrophils show reduced migration *in vitro*,²⁰ but neutrophil morphology in our patients was normal with no excess of non-segmented forms.

In addition to the above abnormality in their neutrophils, our patients with intestinal lymphangiectasia also showed a reduced ability to generate normal chemoattractant activity in their serum, leading to impaired migration by normal cells (Fig. 1). Incubation of serum with zymosan produces activa-

tion of complement via the alternative pathway, with the generation of highly chemoattractant complexes.¹⁰ Serum deficient in complement poorly promotes chemotaxis,^{21 22} but this cannot be the explanation in our patients as complement values were normal.

It has been suggested that fibronectin, a large molecular weight protein found in both circulating and tissue bound forms, may play a part in chemotaxis,²³ and the reduced concentrations of plasma fibronectin in our patients would support this theory. The origin of circulating fibronectin remains uncertain, but as it is a globulin a lymphocyte origin has been suggested,²⁴ and low concentrations might be expected as part of the lymphocyte and immunoglobulin depletion characteristic of intestinal lymphangiectasia.

Impaired serum chemoattractant activity may occur as a result of reduced endogenous stimulation,^{25 26} or may be due to the presence of circulating inhibitors.^{27 28} Chemotactic factor directed inhibitors have been described in association with impaired cell mediated immunity in sarcoidosis,²⁹ leprosy,³⁰ and Hodgkin's disease,³¹ and inhibitors cannot be excluded as the cause of serum defects in our patients.

One of our patients (case 2) developed the additional complication of hyposplenism, which has been reported elsewhere.³² Although defective neutrophil function has yet to be associated with medically acquired hyposplenism, reduced serum chemoattractant activity has been described following surgical removal of the spleen.³³ It has been proposed that this results from reduced concentrations of circulating tuftsin, an immunoglobulin derived tetrapeptide produced in the spleen,³⁴ although this hypothesis has been disputed.³⁵ We did not measure tuftsin concentrations in our patients, but as neither of our other patients showed any evidence of hyposplenism, on the basis of blood film or pitted red cell count,³⁶ tuftsin deficiency seems unlikely to have accounted for our findings and would not explain the additional cell based defect.

Reduced phagocytosis by neutrophils from our patients with intestinal lymphangiectasia, despite the presence of normal serum (Fig. 2), also indicates a cell based defect for this aspect of neutrophil function. The sequences of neutrophil membrane activation leading to particle ingestion are complex and incompletely understood, and our findings do not indicate a site for the observed defect.

Abnormalities of the cell cytoskeleton are known to interfere with phagocytosis in the Chediak-Higashi syndrome,¹⁴ a rare inherited disease associated with recurrent infection, and reduced receptors for immunoglobulin on the neutrophil surface may predispose

to infection in Feltz's syndrome.³⁷ Neutrophils from patients with iron deficiency show reversible defects in phagocytosis and intracellular killing,³⁸ but iron studies in our patients were normal.

There was an additional abnormality in the sera of our patients: they failed to promote normal phagocytosis by control cells (Fig. 2). This defective opsonisation may reflect reduced concentrations of immunoglobulins or fibronectin, as both participate in the coating of particulate matter.³⁹ Immunoglobulins, especially IgG, are important for the binding of coated particles to the neutrophil membrane by receptors specific for the Fc fragment, and synergy has been shown between IgG and complement (C3), enhancing phagocytosis.⁴⁰ Fibronectin promotes bacterial attachment to the neutrophil,⁴¹ but its function in the actual process of phagocytosis has been questioned.⁴⁰⁻⁴² The role of tuftsin in phagocytosis remains uncertain.^{43 44}

Although the full relevance of the observed abnormalities of neutrophil chemotaxis and phagocytosis in patients with intestinal lymphangiectasia requires further evaluation, it seems that impaired neutrophil function may lead to an increased susceptibility to infection in these patients.

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