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Jejunal bacterial overgrowth and intestinal permeability in children with immunodeficiency syndromes

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Abstract

Seventeen paediatric patients with immunodeficiency syndromes (10 with selective IgA deficiency, four with panhypogammaglobulinaemia, and three with selective T cell deficiency) were investigated for bacterial overgrowth of the small intestine and gut permeability to macromolecules. Five of 12 patients showed viable bacterial counts of more than 2×10^5/ml in jejunal fluid. Bacterial overgrowth was also confirmed indirectly by breath hydrogen determination, which was higher than 10 ppm in four of the five patients with positive jejunal culture. Gut permeability to lactulose and L-rhamnose was abnormal in 16 of the 17 immunodeficient patients, who also had higher mean urinary excretion ratios than control subjects – mean (SD) values were 0·216 (0·160) and 0·029 (0·002), respectively. These studies indicate that bacterial overgrowth of the small intestine is a common feature in immunodeficient patients, regardless of the immunological abnormality. Moreover, these patients have an increased gut permeability to macromolecules.

Immunodeficiency syndromes are a heterogeneous group of disorders which include abnormalities of either cell mediated or humoral immunity. Despite the wide spectrum of pathogenic mechanisms that underlie the disorders, however, their clinical features are quite similar – recurrent infections being the hallmark. In paediatric patients, gastrointestinal symptoms are often present, occurring in 50%.[1] These symptoms include chronic or recurrent diarrhoea, which often results in malabsorption syndrome and failure to thrive.[2,3] The severity of symptoms usually correlates with that of the immunological impairment. Combined immunodeficiencies frequently present as intractable diarrhoea, which can be life threatening and often necessitates total parenteral nutrition.[3] The diarrhoea has been associated with histiocytic infiltration of the mucosa[4] and with the persistent and protracted shedding of a wide variety of bacteria, including *Salmonella*, *Shigella*, *Giardia lamblia*, and *Helicobacter* and viruses.[5,6] In most patients the cause of the gastrointestinal involvement remains unknown. Some investigators suggest, however, that colonisation of the small intestine may be associated with nutrient malabsorption or persistent diarrhoea, or both.[7,8] Bacterial overgrowth has already been observed in adults affected by immunodeficiency and is considered to be a consequence of the abnormal secretory immune system.[9]

Another aspect of clinical relevance in immunodeficient patients is the increased incidence of allergic disorders, mainly in those with IgA deficiency syndrome,[10,11] the pathogenic mechanism of which is not well understood. Allergic disorders are interpreted as a consequence of a more complex immunological abnormality affecting the immunoregulatory cells or of gut mucosal damage that allows foreign antigens to reach peripheral blood.[12] Some clinical and laboratory observations favour the latter hypothesis. Raised IgE values[10] and circulating immune complexes containing dietary antigens[13] have been found in IgA deficient patients. Moreover, the presence of whole dietary antigens in the serum of hypogammaglobulinaemic patients with lymphoid hypertrophy has recently been observed.[14] It has also been proposed that longstanding antigenaemia may cause chronic lymphoid stimulation and an increased risk of lymphoma[14] or autoimmune diseases.[15] These findings suggest that secretory antibodies play an important role in preventing entry of luminal antigens in normal subjects.

This study in immunodeficient patients aimed to investigate jejunal bacterial overgrowth and intestinal barrier function, as determined by gut permeation of lactulose and L-rhamnose,[16] to define the relation between gastrointestinal symptoms and bacterial overgrowth, and to clarify the role of abnormal intestinal permeability to predispose such patients to atopic disorders.

Methods

SUBJECTS

A group of 17 patients took part in the study. They had been referred to the department of paediatrics because of clinical evidence of immunodeficiency, consisting of recurrent respiratory and gastrointestinal infections. Laboratory investigations and clinical follow up confirmed a diagnosis of primary immunodeficiency in all patients. Ten were affected by selective IgA deficiency, four by panhypogammaglobulinaemia, and three by selective T cell defect. The major laboratory data are shown in Table I. The patients' age range was 2–17 years. As shown in Table II, 11 patients suffered from recurrent diarrhoea and five from allergic symptoms. No patient had been affected by an acute infectious disease in the 20 days before the study or was receiving antibiotic treatment.

Ten normal age-matched control children (age range 2–15 years), were also tested for intestinal permeability.

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TABLE I *Data in 17 immunodeficient patients*

Diagnosis	E-RFC (%)	CD3 (%)	CD4* (%)	CD8* (%)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
IgA deficiency	81	54	50	20	2390	Abs	244
	61	54	52	20	1917	56	166
	70	64	58	36	1820	Abs	34
	ND	56	48	32	3150	Abs	126
	63	62	58	36	953	32	ND
	69	58	56	42	1280	17	155
	71	60	44	28	2693	Abs	260
	71	55	52	16	665	8	78
	65	52	60	43	2350	Abs	91
	63	ND	47	25	1829	16	248
Hypogammaglobulinaemia	65	53	41	25	205	25	91
	53	50	ND	22	266	31	41
	65	50	32	22	120	16	53
	79	66	39	20	186	Abs	Abs
T cell defect	20	ND	44	26	855	43	45
	31	34	36	32	1511	244	290
	16	30	40	30	1030	79	238

E-RFC=E rosette forming cells; *=positive cells on T enriched suspensions; Abs=absent; ND=not determined.

Informed parental consent was obtained before testing the children.

BACTERIOLOGICAL STUDIES

Samples of jejunal juice were obtained after overnight fast by aspiration via a peroral sterile radio-opaque tube, which was guided beyond the Treitz flexure under radiological control. The first 5 ml of aspirate were discarded. The samples were cultured in either oxygen free atmosphere for anaerobic bacteria or for aerobic micro-organisms, as previously described in detail.[17 18] In brief, 10% blood agar and MacConkey agar were used for the identification of aerobes, Sabouraud agar was used for yeasts, and Wilkins-Chargrel agar for anaerobes. Samples were also examined by light microscopy for the presence of *Giardia lamblia*. For ethical reasons, this technique was not performed in normal subjects.

BREATH HYDROGEN CONCENTRATION

The breath hydrogen concentration was measured by a gas chromatograph (HP 5840 Hewlett-Packard Instruments, Avondale, Palo Alto, USA) either 20 minutes after an oral load with a 10% aqueous solution of glucose (2 g/kg body weight) or after overnight fast.[19 20] All patients received both a fasting and a glucose challenge test.

TABLE II *Gastrointestinal and allergic symptoms in 17 immunodeficient patients*

Diagnosis	Age (yrs)	Recurrent diarrhoea	Failure to thrive	Asthma	Eczema
IgA deficiency	12	+	−	−	−
	5	+	−	−	−
	11	+	−	−	−
	9	+	−	+	−
	2	+	−	−	−
	6	−	−	−	−
	5	−	−	−	−
	17	−	−	−	−
	5	+	−	−	−
	6	−	−	−	−
Hypogammaglobulinaemia	14	+	−	−	−
	5	+	−	+	−
	3	+	−	−	+
	2	−	−	−	−
T cell defect	3	+	+	+	−
	10	+	+	−	+
	2	−	−	−	−

INTESTINAL PERMEABILITY

The study of permeability was performed according to Parrilli *et al.*[21] An oral loading solution was given to the patients and controls after overnight fast. The aqueous solution (340 mmol/l) contained 5 g of lactulose (Duphalac, Duphar) and 0·5 g of L-rhamnose (Sigma). Urine was collected for five hours after the oral load and a sample was preserved with 0·1 mg/ml merthiolate and stored at −20°C for sugar analysis. Thin layer chromatography was used to determine the urinary sugar content.[22] Results were expressed as the urinary recovery percentage of the oral dose.

STATISTICAL ANALYSIS

The significance of the differences was calculated by Student's *t* test. A 2×2 contingency table and Fisher's exact test were also used for the breath hydrogen concentration test.

Results

CLINICAL FINDINGS

The clinical findings are summarised in Table II. Eleven patients suffered from recurrent diarrhoea or failure to thrive (weight/height below the fifth centile) and five had asthma or eczema.

BACTERIOLOGICAL STUDIES

More than 2×10^5 organisms/ml were isolated in five of the 12 patients (41%) who underwent culture of jejunal fluid (five patients refused the study). They consisted of Gram positive oral-type bacteria. *Streptococci* and *Staphylococci* clearly predominated. This would be expected in bacterial overgrowth not caused by intestinal obstruction, in which enteric coliform bacteria are predominant. All the three immunodeficiency syndromes were represented in the group of positive patients – one had a selective T cell defect, one had hypogammaglobulinaemia, and the remaining three patients had IgA deficiency. No sample was positive for anaerobes or *Giardia lamblia*.

BREATH HYDROGEN CONCENTRATION

The breath hydrogen concentration after fasting or glucose load, or both, was higher in the five patients with bacterial overgrowth than in the remaining seven patients with negative jejunal fluid culture: mean (SD) values were 10·82 (7·01) ppm 3·92 (5·70) ppm, respectively ($0{\cdot}1>p>0{\cdot}05$). Taking a breath hydrogen concentration of 10 ppm as a cut off point, four of the five patients with bacterial overgrowth had a value higher than this. In contrast, only one of the patients without bacterial overgrowth showed a higher concentration than 10 ppm ($p<0{\cdot}05$). Two of the patients with bacterial overgrowth and a high breath hydrogen concentration showed an increase in fasting breath hydrogen only whereas the remaining three showed an increase in both fasting and post glucose load values.

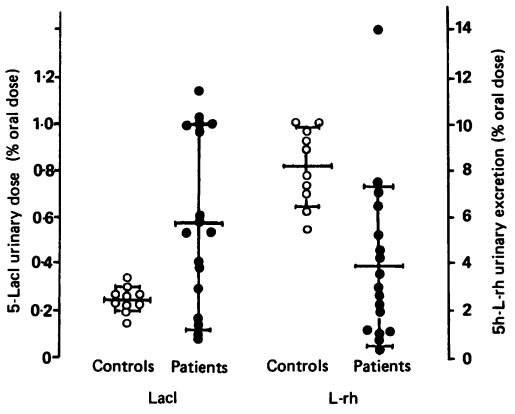

Figure 1: Five hour lactulose (Lacl) and L-rhamnose (L-rh) urinary excretion expressed as a % of oral dose in patients (closed circles) and controls (open circles). The bars indicate mean (SD) value.

INTESTINAL PERMEABILITY

The five hour urinary excretion of lactulose and L-rhamnose in 10 age-matched control subjects, expressed as mean (SD) values of oral dose percentages, were 0·25 (0·05)% and 8·2 (1·7)%, respectively. Lactulose excretion (Fig 1) in patients was 0·58 (0·46)% (patients *v* controls: $p<0·01$). In the patient group the L-rhamnose excretion (Fig 1) was lower than in controls (mean (SD) 3·92 (3·45)%, $p<0·001$). Lactulose/L-rhamnose excretion ratios (Fig 2) in patients and controls were 0·216 (0·160) and 0·029 (0·002) respectively ($p<0·001$). Lactulose/L-rhamnose excretion ratios were not significantly different in the three subgroups of patients.

CORRELATION BETWEEN CLINICAL, BACTERIOLOGICAL, AND GUT PERMEABILITY FINDINGS

All patients with gastrointestinal or atopic symptoms showed abnormal intestinal permeability. All 12 patients who were also tested for bacterial overgrowth were symptomatic. Five of these had both bacterial overgrowth and an abnormal intestinal permeability. Four of the five patients with bacterial overgrowth had shown gastrointestinal symptoms.

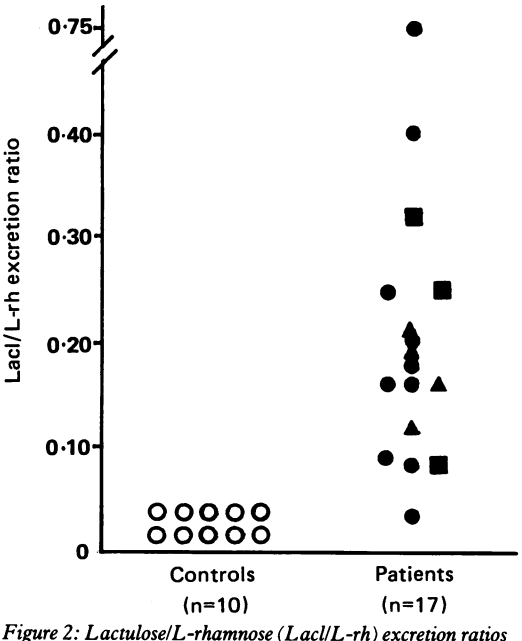

Figure 2: Lactulose/L-rhamnose (Lacl/L-rh) excretion ratios in controls (○) and in patients affected by IgA deficiency (●), panhypogammaglobulinaemia (▲) and T cell defect (■).

Discussion

In this study five of 12 immunodeficient patients (41%) were shown to have bacterial overgrowth of the small intestine by culture of the jejunal fluid. This finding was also confirmed indirectly in four of these five by hydrogen breath test, which showed a slight increase in the fasting or post glucose load values, or both. Bacterial overgrowth was not related to the nature of the immunological abnormality, it was present in IgA deficient patients, in a panhypogammaglobulinaemic subject, and in a patient with selective T cell defect. The satisfactory correlation between the hydrogen breath test values and bacterial overgrowth supports the hypothesis that the former represents a useful non-invasive tool by which to screen these patients.

In sixteen of the 17 patients increased urinary excretion of lactulose and reduced absorption of L-rhamnose was observed, together with a sevenfold increase in the urinary lactulose/L-rhamnose ratio. Lactulose is a disaccharide whose transmucosal transfer follows paracellular pathways (intercellular tight junctions or cell extrusion zones, or both).[23] The increased lactulose excretion after oral glucose load possibly reflects a reduced barrier function in the gut. A similar alteration has also been reported in patients with coeliac disease,[24] which is associated with appreciable malabsorption of monosaccharides such as D-xylose and L-rhamnose and with an abnormal increase in intestinal permeation of lactulose. Our observation favours the hypothesis that immunodeficient patients, regardless of the immunological abnormality, frequently have gastrointestinal mucosal lesions, possibly patchy in nature, that may be responsible for nutrient malabsorption and allow permeation of foreign antigens. High values of dietary bovine antigens have, in fact, been observed in panhypogammaglobulinaemic patients.[14]

There is not a conclusive explanation for the pathogenesis of this mucosal damage. The presence of both bacterial overgrowth and abnormal intestinal permeability in 41% of the patients may, however, suggest a causal relation between the two.

Two main considerations arise from our data: firstly, the intestinal microenvironment is under the control of either the secretory IgA mediated system or the other immunological systems involving non-IgA antibodies and T lymphocytes that play an important role in maintaining the homeostasis of the internal environment. This role probably influences the intestinal microflora directly and the exclusion of dietary antigenic constituents indirectly.

Looked at in another way, the integrity of the gastrointestinal mucosa is necessary to avoid a further deterioration of the immune system due to malnutrition.

Our data may have some implications for treatment. Bacterial overgrowth should be sus-

pected in all patients with immunodeficiencies, and treatment started early since increased viable counts of oral-type bacteria in the jejunum may have a possible role in recurrent diarrhoea. As for the increased gut permeability, controlled trials should be undertaken to prove the efficacy of elemental diets in the supportive treatment of severe illness to prevent the penetration of whole foreign antigens into the blood.

In conclusion, the aim of the present study is to stress the importance of the gastrointestinal aspects in the general management of immunodeficient patients and to contribute to knowledge of the pathogenesis of some complications of immunodeficiency disorders.

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