Jejunal mucosal enzyme activities, regulatory peptides and organelle pathology of the enteropathy of common variable immunodeficiency

J DAWSON, M G BRYANT,* S R BLOOM, AND T J PETERS

From the Division of Clinical Cell Biology, MRC Clinical Research Centre, Harrow, Middlesex and Department of Medicine, Royal Postgraduate Medical School, London

SUMMARY Jejunal biopsies from six patients having the small bowel enteropathy associated with common variable immunodeficiency have been subjected to analytical subcellular fractionation and enzymic and regulatory peptide microassay to define the organelle pathology of this syndrome. Compared with normal subjects, the immunodeficient patients had decreased activities of the three brush border enzymes: alkaline phosphatase, γ -glutamyl transferase and α -glucosidase. The other organelle marker enzyme activities and all the regulatory peptide concentrations did not differ from the controls. Density gradient experiments showed a complete loss of particulate β -glucosidase (lactase) with activity entirely located in the cytosol. The integrity of other organelles was normal. These data indicate that the enteropathy of common variable immunodeficiency is associated with abnormalities in the jejunal brush border analogous to those present in tropical malabsorption syndrome.

Gastrointestinal symptoms are found in up to 60% of patients with common variable immunodeficiency (idiopathic acquired hypogammaglobulinaemia).¹ The cause of these symptoms is probably multifactorial but malabsorption is often prominent and certain jejunal histological abnormalities have been described and compared with those found in coeliac disease and tropical malabsorption.²⁻⁴ The biochemical abnormalities found in the mucosa in these two diseases are, however, distinct, probably reflecting the different pathogenesis of the mucosal damage.⁵⁻⁷ Furthermore, the mucosal regulatory peptides show characteristic abnormalities in these two forms of enteropathies.^{8,9} There are, however, no reports of investigations into the pathogenesis of the enteropathy of common variable immunodeficiency at the cellular and subcellular level. In this study we have assayed the mucosal enzyme activities and regulatory peptide concentrations and investigated the intracellular organelles in the jejunum from six patients with common variable immunodeficiency undergoing investigation for profuse diarrhoea.

Methods

PATIENTS

Six patients, five men and one woman, with common variable immunodeficiency, age 19-31 years, were studied. All had diarrhoea in excess of 500 g daily with documented steatorrhoea and had jejunal biopsies showing variable partial villous atrophy (Fig. 1). Two patients had *Giardia lamblia* infestation. None were receiving specific therapy (other than regular immunoglobulin injections) or dietary measures at the time of the study. Six healthy adult subjects without diarrhoea served as controls. Crosby capsule biopsies were obtained from the jejunum, 6 cm from the ligament of Trietz, and the specimen was divided into two portions. One was placed in formalin solution and processed for histology. The other portion, approximately 10 mg wet weight, was transferred to ice cold sucrose (0.3 mol/l) containing 1 mmol/l disodium EDTA and 22 mmol/l ethanol (sucrose medium). Tissue was homogenised as described previously¹⁰ and, after low speed centrifugation to remove the nuclei and cell debris, the postnuclear supernatant was subjected to isopycnic sucrose density centrifugation in a Beaufay small volume automatic zonal rotor, as previously described.^{10,11} The rotor was unloaded

Address for correspondence: Professor T J Peters, Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ.

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*Deceased

automatically and some 15 fractions were collected in tared tubes. After reweighing and mixing, the densities of the fractions were determined indirectly with an Abbé refractometer.

Enzyme activities of gradient fractions, post-nuclear supernatant and nuclear fractions were determined by micromethods previously described.¹¹ The results were expressed as milliunits of enzyme activity per mg biopsy protein where one unit is equal to 1 μ mol of substrate transformed per minute. Aliquots of the fractions were mixed with equal volumes of 0.2 mol/l HCl and frozen before assay for gastrin, GIP, motilin, secretin, somatostatin, enteroglucagon and VIP using previously validated radio immunoassays especially modified and optimised for the small quantities of tissue available.^{10 12-15} Peptide concentrations were expressed as pmol/mg of biopsy protein. Protein content of the homogenate was assayed by the Lowry technique.¹⁶ Protein in the subcellular fractions was assayed by a micro-modification¹⁷ of the fluorimetric technique of Hiroaka and Glick¹⁸ with bovine serum albumin (Sigma, London) as standard. The enzyme and hormone distribution results were expressed as frequency density histograms. All calculations, plots and fractionation recoveries were performed by computer as described previously.¹¹ These studies were approved by the local ethical committee.

Results

The Table shows the organelle marker enzyme activities and the mucosal peptide concentrations in the patients with common variable immunodeficiency. Alkaline phosphatase, γ -glutamyl transferase and α -glucosidase (pH 6.0) (brush border enzymes) are significantly reduced in comparison with normal controls. All other enzymes had similar activities to the controls. No significant differences from normal controls were noted in mucosal peptide concentrations. It is unlikely that the modest increase in mucosal lymphocytes would contribute to the total protein content of the biopsy expressed/mg wet weight.

Figures 2 and 3 compares the density distribution of 12 organelle marker enzymes in the patients with control subjects. The most obvious difference in the patients was a lack of particulate β -glucosidase. It was also noted that there was a more prominent brush border component of 5'-nucleotidase and a broadening of the peak of acid phosphatase. The other organelles were similar to the normal controls. No differences were noted between the patients with and without *Giardia* infestations.

Discussion

This paper is the first attempt to systematically

Table Enzyme activities and peptide concentrations in jejunal homogenates in common variable immunodeficiency

Enzyme/Peptide	EC no	Specific activity/concentration	
		Controls	Common variable immunodeficiency
Alkaline phosphatase	3.1.3.1	28.4 $\pm$ 6.2	14.6 $\pm$ 2.3*
γ -Glutamyl transferase	2.3.2.2	4.12 $\pm$ 1.10	1.19 $\pm$ 1.20*
α -Glucosidase (pH 6.0)	3.2.1.20	6.21 $\pm$ 0.72	2.06 $\pm$ 1.40*
Catalase	1.11.1.6	40.4 $\pm$ 3.2	41.3 $\pm$ 4.3
N-Acetyl- β -glucosaminidase	3.2.1.30	2.38 $\pm$ 0.31	2.41 $\pm$ 0.47
Acid phosphatase	3.1.3.2	16.2 $\pm$ 1.6	12.6 $\pm$ 1.4
β -Glucosidase	3.2.1.21	0.61 $\pm$ 0.08	0.62 $\pm$ 0.07
Malate dehydrogenase	1.1.1.37	1390 $\pm$ 280	1490 $\pm$ 360
Lactate dehydrogenase	1.1.1.27	162 $\pm$ 28	187 $\pm$ 57
α -Glucosidase (pH 8.0)	3.2.1.20	0.64 $\pm$ 0.21	0.85 $\pm$ 0.45
5'-Nucleotidase	3.1.3.5	16.4 $\pm$ 1.4	18.1 $\pm$ 3.5
Gastrin		2.6 $\pm$ 0.4	2.1 $\pm$ 0.5
Gastrin inhibitory polypeptide (GIP)		2.5 $\pm$ 0.4	2.9 $\pm$ 0.6
Enteroglucagon		0.6 $\pm$ 0.2	0.4 $\pm$ 0.1
Motilin		3.9 $\pm$ 0.7	3.6 $\pm$ 0.3
Secretin		3.1 $\pm$ 0.6	4.1 $\pm$ 0.7
Somatostatin		9.4 $\pm$ 1.6	12.4 $\pm$ 0.9
Vasoactive inhibitory polypeptide (VIP)		1.9 $\pm$ 0.6	2.7 $\pm$ 0.4
Total protein (mg)		3.1 $\pm$ 0.4	2.4 $\pm$ 0.4

Enzyme activities expressed as munits/mg protein (mean $\pm$ SE). Peptide concentrations expressed as pmol/mg protein (mean $\pm$ SE). Statistical analysis by Student's *t* test: **p*<0.05.

Fig. 2 Isopycnic centrifugation of post-nuclear supernatant from jejunal biopsy homogenate from patients with common variable immunodeficiency (solid line) and normal controls (dotted line). Graphs show frequency-density histograms for organelle marker enzymes and represent averaged data from six patients and six controls. Frequency (mean $\pm$ SD) is defined as the fraction of total recovered activity present in the individual fraction divided by the density span covered by that fraction. The activity present over the density span $1.05\text{--}1.10\text{ g cm}^{-3}$ represents, over an arbitrary abscissa interval, activity remaining in the sample layer and is presumed to reflect soluble activity. Percentage recoveries (mean $\pm$ SD) were: lactate dehydrogenase, 87 ± 19 ; α -glucosidase pH 8.0, 92 ± 18 ; 5'-nucleotidase, 102 ± 18 ; malate dehydrogenase, 88 ± 20 ; β -glucosidase, 97 ± 13 ; protein, 89 ± 19 .

investigate the jejunal mucosal enzymes and the organelle pathology in common variable immunodeficiency. Patients with common variable immunodeficiency show decreased activities of the three brush border enzymes studied but all other marker enzymes had activities similar to normal controls. This pattern of abnormality is similar to that found in the tropical malabsorption syndrome.⁷ It is noted that, as in tropical malabsorption,¹⁹ patients with common variable immunodeficiency have patchy jejunal histological abnormalities of the partial villous atrophy type and a high incidence of infestation with *Giardia lamblia*.^{1,2} In the present study all patients had partial villous atrophy and two were

Fig. 3 Isopycnic centrifugation of post-nuclear supernatant from jejunal biopsy homogenate from patients with common variable immunodeficiency (solid line) and normal controls (dotted line). Graphs show frequency-density histograms for organelle marker enzymes and represent averaged data for six patients and six controls. For details see legend to figure 1. Percentage recoveries (mean $\pm$ SD) were: alkaline phosphatase, 102 ± 12 ; γ -glutamyl transferase, 97 ± 14 ; α -glucosidase (pH 6.0), 98 ± 18 ; catalase, 101 ± 12 ; N-acetyl- β -glucosaminidase, 89 ± 18 ; acid phosphatase, 92 ± 15 .

infested with giardia. It is likely therefore that the brush border changes in the immunodeficiency patients and in tropical malabsorption have a common pathogenesis, and that the changes are secondary to occult or proven infection. Whether the mucosal architectural changes are because of infection, as has been suggested on clinical grounds,^{2,3} remains speculative.

The major abnormality of the organelle marker enzymes in the density gradient experiments was that of β -glucosidase. This enzyme has a complex localisation in normal jejunum to cytosol, brush border and endoplasmic reticulum,¹¹ but in this disorder there is a loss of both particulate peaks and the activity is almost entirely soluble. A similar phenomenon is noted in coeliac disease, where it has been suggested to be of pathogenic significance.⁶ The present study, however, has shown that the lysosomal enzyme changes characteristic of coeliac disease³ are not found in common variable immunodeficiency. Because changes in this organelle are more likely to be involved in the pathogenesis of the more profound mucosal lesion of coeliac disease it is

probable that the changes in β -glucosidase noted in the present study are secondary to the mucosal damage.

Mucosal regulatory peptide concentrations were noted to be normal. Although there are no reports of plasma hormone release in patients with common variable immunodeficiency, it is known that basal plasma gastrin concentrations are normal, an observation which has been used to differentiate the vitamin B12 malabsorption in this condition from true pernicious anaemia where the plasma gastrin is raised.²⁰ There is a previous single case report in which jejunal endocrine cells were noted to be normal in common variable immunodeficiency.²¹ This, together with the normal mucosal levels, found in the present study serves further to emphasise the difference in the mucosal lesion in this condition and coeliac disease where marked abnormality of endocrine cells^{22, 23} and mucosal regulatory peptide concentrations⁹ are noted.

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