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Faecal interleukin-8 and tumour necrosis factor-α concentrations in cystic fibrosis

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Abstract

Interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) concentrations were measured in faecal samples from nine patients with cystic fibrosis and nine healthy age matched controls. The patients were assessed with Shwachman score, apparent energy absorption, pancreatic enzyme dosage, simple spirometry, and presence of pseudomonal colonisation. Median (range) wet stool IL-8 and TNF-α concentrations in patients were 32 113 pg/g (21 656–178 128) and 3187 pg/g (368–17 611) respectively, compared with <43·5 pg (IL-8)/g (<22–4079) and 99 pg (TNF-α)/g (<0·26–231) in controls. IL-8 concentration was negatively correlated with Shwachman score ($r=-0·79$) and pancreatic enzyme dosage ($r=-0·77$), but not with energy absorption. Seven patients were mature enough to cooperate with spirometry. Their IL-8 concentrations correlated with percentage predicted forced expiratory volume in one second ($r=-0·78$). IL-8 concentration was greater in four patients with, than five without, established pseudomonal colonisation: median difference 134 583 pg/g. TNF-α concentration was not correlated with measures of disease severity. Faecal IL-8 concentration might reflect the severity of pulmonary inflammation in cystic fibrosis and could provide an easily obtainable marker of disease activity.

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Interleukin-8 (IL-8) is strongly chemotactic for neutrophil polymorphs and is responsible for the continued neutrophil migration into the airways that characterises chronic pulmonary disease in cystic fibrosis.[1] Lower respiratory tract secretions are cleared by coughing and by the mucociliary escalator. Both result in respiratory secretions being swallowed. In patients with cystic fibrosis receiving pancreatic enzyme replacement treatment for pancreatic insufficiency, digestion may be incomplete. We hypothesised that IL-8, a protein of 8·3 kilodaltons, which is relatively resistant to acidity and proteases, may pass through the cystic fibrosis intestine undigested and may be present in the stool in measurable quantities. If this hypothesis were correct, and if faecal IL-8 did not originate from elsewhere in the gastrointestinal tract, its measurement might reflect daily pulmonary IL-8 production. Such an index of pulmonary inflammation could be useful, particularly in patients too young to expectorate.

Faecal cytokine concentrations (tumour necrosis factor-α (TNF-α) and IL-6) have been measured in inflammatory bowel disease and infectious diarrhoea and in those studies, TNF-α concentration correlated with bowel inflammatory activity.[2–4] Here we report the first assays of faecal IL-8 and TNF-α concentration in patients with cystic fibrosis.

Subjects and methods

PATIENTS AND CONTROLS

We collected 24 hour stool samples from nine pancreatic insufficient patients with cystic fibrosis. Their pancreatic enzyme preparation was Creon 25000 (Duphar), except for patient 1 (Creon, Duphar) and patient 7 (Nutrizym 22, Merck). These faecal samples had been collected for another study investigating nutrient digestion and absorption. Control samples were collected from clinically healthy children in the community. They were age matched and paired with patients before analysis of the samples.

Four patients (1–4) had established pseudomonal colonisation and had suffered exacerbations of chest disease. Two patients (5 and 8) had never grown pseudomonas, and two patients (9 and 6) had grown it on a single occasion from a throat swab one and two years before the study respectively. Patient 7 had isolated pseudomonas from a throat swab twice: two years and then two months before the study. No study patient had ever been culture positive for *Burkholderia cepacia*. Patient 3 had cirrhosis and portal hypertension; no other patient had liver disease. No control had any concurrent disorder.

METHODS

Stool samples were homogenised to a manageable consistency with water. Aliquots of the homogenate were frozen at −20°C and stored. The homogenised stool was thawed and 1 g was added to 1 ml of phosphate buffered saline. This was vortexed for two minutes and centrifuged at 20 000 *g* for 15 minutes at 4°C. The aqueous supernatant was removed and frozen at −70°C until analysis. The IL-8 assay has been described elsewhere.[5] TNF-α was assayed using the Quantikine kit (R and D Systems) according to the manufacturer's instructions. All cytokine concentrations were expressed as pg/g of wet stool.

The patients were assessed by Shwachman score, apparent energy absorption, pancreatic

Table 1 Patients' characteristics

Patient No	*Shwachman score*	*Lipase dosage**	*% Energy absorption*	*FVC (% predicted)*	*FEV_1 (% predicted)*	*FEF_{25-75} (% predicted)*
1	47	7·7	87·2	52	22	7
2	71	4·3	81·5	56	29	10
3	91	23·5	83·6	76	70	52
4	76	10·2	91·2	91	53	19
5	97	20·8	88·7	62	59	47
6	95	25·7	85·6	99	84	47
7	92	37·0	61·3	–	–	–
8	94	31·3	89·6	–	–	–
9	90	18·6	92·4	87	74	44

*Thousands of European lipase units/kg body weight/day.

enzyme dosage, and, in those old enough to cooperate, by spirometry. Weighed food intake was recorded for five days as described by Marr[6] and gross energy intake calculated.[7] A three day stool collection between carmine markers was homogenised and faecal energy was measured in a ballistic bomb calorimeter. Apparent energy absorption was expressed as a percentage of intake. Comparative dosages of pancreatic enzymes were expressed as thousands of European units of lipase activity/kg body weight/day. Shwachman scores were calculated at a full clinical review in advance of the assays.

Results

Patients are described in table 1, and faecal cytokine concentrations are shown in table 2. The median (range) stool IL-8 and TNF-α concentrations in patients was 32 113 pg/g (21 656–178 128) and 3187 pg/g (368–17 611) respectively. In controls cytokine concentration was below the detection limit of the assay in seven of nine IL-8 samples and two of nine TNF-α samples. Assuming the respective concentrations to be at their detection limits, the median (range) stool IL-8 and TNF-α concentrations in controls are estimated at <43·5 pg/g (<22–4079) and 99 pg/g (<0·26–231).

The median (95% confidence interval (CI)) difference in stool cytokine concentrations (cystic fibrosis population minus population without cystic fibrosis) was 87 072 pg/g (25 090 to 154 388) for IL-8 and 3705 pg/g (925 to 10 267) for TNF-α. Faecal cytokine concentration was greater in patients with, than in those without, established pseudomonal colonisation: median difference 134 583 pg (IL-8)/g and 5740 pg (TNF-α)/g.

In patients, IL-8 concentration was negatively correlated with Shwachman score ($r=-0·79$, 95% CI −0·95 to −0·27, $p<0·02$, figure) and lipase usage ($r=-0·77$, 95% CI −0·95 to −0·21, $p<0·02$), but not with energy absorption. Seven patients were able to perform simple spirometry. Faecal IL-8 concentration was negatively correlated with forced expiratory volume in one second (FEV_1) ($r=-0·78$, 95% CI −0·97 to −0·06, $p<0·05$). There was good correlation between IL-8 concentration and the forced vital capacity (FVC) ($r=-0·57$) and forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75}) ($r=-0·73$), but the small sample size prevented these coefficients from reaching statistical significance. Faecal TNF-α concentrations did not correlate with lung function, Shwachman score, lipase usage, or energy absorption.

Six months after the study, patient 7 became consistently colonised with pseudomonas, on the basis of repeated throat swab isolates and increased symptoms. He was receiving 54 000 units of lipase/kg/day and faecal analysis after one week of intravenous antibiotic showed little change in cytokine concentrations: 22 562 pg/g (IL-8) and 2052 pg/g (TNF-α). Four months later he presented with a splenic flexure colonic stricture.[8] A child selected as his control (age 5·2 years) had asthma, was receiving sodium cromoglycate, and was asymptomatic. His faecal concentrations were 9713 pg/g (IL-8) and 247 pg/g (TNF-α).

Table 2 Faecal IL-8 and TNF-α concentrations

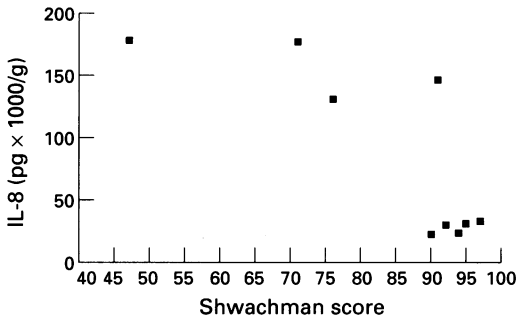
	Controls			*Patients*		
No	*Age (years)*	*IL-8*	*TNF-α*	*Age (years)*	*IL-8*	*TNF-α*
1	13·7	<33	66	12·9	178 128	3 187
2	21·6	<22	37	20·6	176 497	5 897
3	9·5	<31·5	<0·26	9·7	146 438	17 611
4	15·2	<43·3	155	14·3	130 725	10 422
5	7·6	496·2	<0·68	3·7	32 113	4 472
6	11·5	<53·7	123	7·4	30 439	1 075
7	5·1	4079·3	129	3·9	29 169	1 497
8	7·9	<56·6	231	5·3	22 872	368
9	9·7	<43·5	99	7·8	21 656	998

Cytokine concentrations are expressed as pg/g of wet stool.

Discussion

We have shown raised faecal IL-8 and TNF-α concentrations in patients with cystic fibrosis, when compared with healthy children. Differences in cytokine concentration between disease and control groups were greater for IL-8 than TNF-α when they were expressed weight for weight. These differences are even greater when expressed in molar units, given the small molecular weight of IL-8.

TNF-α concentrations in our control samples are comparable with those of the normal control children used by Nicholls *et al* (12–130 pg/g)[3] and by Braegger *et al* (40–84 pg/g).[2] The faecal TNF-α concentrations in our cystic fibrosis patients are similar to those of active ulcerative colitis and patients with Crohn's disease. None of our patients had inflammatory bowel disease clinically, but we cannot exclude subclinical intestinal inflammation, not having taken biopsy specimens. Indeed our patient who subsequently developed a colonic stricture did have a mild chronic inflammatory infiltrate at the time of his colectomy. However, his IL-8 concentrations were only moderately raised. If faecal cytokines originate from chronic inflammation accompanying colonic stricture we would expect to have seen more strictures in our study population. These strictures have been associated with high lipase pancreatic enzymes.[9] In our study only one of the patients was taking low strength pancreatic enzyme, and his faecal IL-8 concentration was the highest we recorded. The negative correlation between faecal IL-8 concentration and pancreatic enzyme dosage does not support the



Shwachman score and faecal IL-8 concentration.

idea that the enzyme causes subclinical intestinal inflammation. It is possible that a neutrophil infiltrate occurs in the bowel in cystic fibrosis as a result of the high lumenal IL-8 concentration, if this IL-8 is biologically active. Potentially lumenal IL-8 could be the cause rather than the result of subclinical intestinal inflammation.

Sheron *et al* have demonstrated raised IL-8 concentrations in the serum and liver tissue of adults with acute severe alcoholic hepatitis which is characterised by an intense tissue neutrophilia.[10] Hepatic tissue concentrations in that group of patients were one to two orders of magnitude greater than the faecal concentrations we have recorded. If hepatic tissue IL-8 concentration in liver disease in cystic fibrosis was comparable with that in acute severe alcoholic hepatitis, liver disease could be a major source of faecal cytokines. While we cannot discount the possibility that hepatic disease may contribute to stool IL-8 concentrations, it is unlikely to be its major source as eight of our nine patients had no evidence of liver disease. An alternative source for these high cytokine concentrations must be postulated.

The large difference in IL-8 concentration between patients with and without established pseudomonal colonisation and the negative correlation of Shwachman score and lung function with faecal IL-8 concentration are consistent with a pulmonary source for this cytokine.

Greally *et al* have described TNF-α concentrations between 10 and 1988 pg/ml in the sputum of 16 patients with cystic fibrosis.[11] The faecal TNF-α concentrations in our patients are slightly higher (368–17 611 pg/g), although they are expressed differently. We have previously shown high IL-8 concentrations in cystic fibrosis sputum, serum, and bronchoalveolar lavage fluid,[5] with concentrations between 621 and 92 628 pg/g wet weight of sputum. Faecal concentrations in the present study are in excess of this using an identical assay. If TNF-α and IL-8 simply pass through the gastrointestinal tract, the high concentrations in the dietary residue imply that the largest part of daily sputum production is swallowed rather than expectorated, and that volumes of sputum are much higher than has been appreciated, even in patients without overt chest symptoms. It is possible that faecal cytokine concentration reflects the severity of pulmonary inflammation if it is primarily influenced by both sputum volume and its cytokine content. Our results suggest that faecal IL-8 concentration is more likely than TNF-α concentration to reflect severity of pulmonary inflammation.

Sputum entering the upper gastrointestinal tract will contain IL-8, TNF-α, inflammatory cells, and organisms. The cellular content of swallowed sputum may continue to produce cytokines as it passes through the bowel. It may stimulate intestinal cells to secrete them or they may be secreted in response to swallowed organisms. All three mechanisms would allow for amplification of lumenal cytokine concentration, making it an indirect index of pulmonary inflammation.

The possibility that faecal IL-8 concentration may reflect severity of pulmonary inflammation in cystic fibrosis deserves further consideration because the test is easily repeated, non-invasive, and would be applicable to patients who are too young to expectorate sputum. It may even be of use in other suppurative lung diseases.

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1 Kunkel SL, Standiford T, Kasahara K, Strieter RM. Interleukin 8 (IL-8): the major neutrophil chemotactic factor in the lung. *Exp Lung Res* 1991; **17:** 17–23.
2 Braegger CP, Nicholls S, Murch SH, Stephens S, MacDonald TT. Tumour necrosis factor alpha in stool as a marker in intestinal inflammation. *Lancet* 1992; **339:** 89–91.
3 Nicholls S, Stephens S, Braegger CP, Walker-Smith JA, MacDonald TT. Cytokines in stools of children with inflammatory bowel disease or infective diarrhoea. *J Clin Pathol* 1993; **46:** 757–60.
4 Harendra de Silva DG, Mendis LN, Sheron N, *et al.* Concentrations of interleukin 6 and tumour necrosis factor in serum and stools of children with Shigella dysenteriae 1 infection. *Gut* 1993; **34:** 194–8.
5 Dean TP, Dai Y, Shute JK, Church MK, Warner JO. Interleukin-8 concentrations are elevated in bronchoalveolar lavage, sputum and sera of children with cystic fibrosis. *Pediatr Res* 1993; **34:** 159–61.
6 Marr JW. Individual dietary surveys: purposes of methods. *World Rev Nutr Diet* 1971; **13:** 105–64.
7 Murphy JL, Wootton SA, Bond SA, Jackson AA. Energy content of stools in normal healthy controls and patients with cystic fibrosis. *Arch Dis Child* 1991; **66:** 495–500.
8 Briars GL, Griffiths DM, Moore IE, Williams P, Johnson K, Rolles CJ. High strength pancreatic enzymes. *Lancet* 1994; **343:** 600.
9 Smyth RL, Van Velzen D, Smyth AR, Lloyd DA, Heaf DP. Strictures of ascending colon in cystic fibrosis and high strength pancreatic enzymes. *Lancet* 1994; **343:** 85–6.
10 Sheron N, Bird G, Koskinas J, *et al.* Circulating and tissue levels of the neutrophil chemotaxin interleukin-8 are elevated in severe acute alcoholic hepatitis and tissue levels correlate with neutrophil infiltration. *Hepatology* 1993; **18:** 41–6.
11 Greally P, Hussein MJ, Cook AJ, Sampson AP, Piper PJ, Price JF. Sputum tumour necrosis factor-α and leukotriene concentrations in cystic fibrosis. *Arch Dis Child* 1993; **68:** 389–92.