Serum concentrations of tumour necrosis factor α in childhood chronic inflammatory bowel disease

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Abstract

Serum tumour necrosis factor α (TNFα) concentrations were measured by enzyme linked immunoadsorbent assay in 31 normal children and during 65 episodes of clinical remission and 54 episodes of relapse in 92 children with chronic inflammatory bowel disease. An appreciable rise in TNFα was found only in children in relapse of ulcerative colitis and colonic Crohn's disease. The group of children with small bowel Crohn's disease in relapse did not show increases of TNFα above control concentrations, despite an equivalent rise in disease indices. Height velocity was depressed in children with relapse of large bowel Crohn's disease and ulcerative colitis compared with the equivalent condition in remission. The impairment of growth velocity was significantly greater in relapse of large bowel Crohn's disease and ulcerative colitis than in small bowel Crohn's disease alone, although for the subgroups in stage 1 puberty (prepubertal) the differences were not significant. Inadequate growth in chronic inflammatory bowel disease is currently ascribed to inadequate nutrition and TNFα may contribute to this through its cachexia inducing effects. It may, in addition, diminish pituitary growth hormone release. These results suggest that production of TNFα may be associated with growth failure in relapse of colonic inflammatory bowel disease.

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Tumour necrosis factor α (TNFα)/cachectin, a cytokine produced mainly by activated macrophages and monocytes, is generating increasing interest both as a mediator of tissue maturation and local immunity and as a destructive agent capable of causing profound cachexia and tissue injury. The balance of beneficial and deleterious effects are determined by its level of production and its interaction with other mediators.[1-4]

Excess production of TNFα has been found in parasitic diseases including trypanosomiasis and malaria[5] (in which it may be a major mediator of cerebral involvement[6]), in malignancy,[7] connective tissue diseases,[8] and septicaemia.[9] High serum concentrations have been shown to be associated with poor outcome in meningococcal septicaemia[10] and raised concentrations in cerebrospinal fluid have been shown in bacterial meningitis in childhood.[11]

There have been no studies in which TNFα concentrations have been measured in childhood chronic inflammatory conditions, and it has not previously been implicated in growth failure.

Methods

PATIENTS

Control subjects
Twenty five of the 31 children used as control subjects underwent colonoscopy and barium followthrough examination for investigation of possible inflammatory bowel disease. In all cases full investigation was normal and a diagnosis of functional abdominal pain or irritable bowel syndrome was made. Biopsy specimens showed no evidence of microscopic colitis, barium followthrough was normal or showed lymphoid nodular hyperplasia only (five cases) and there was no rise in erythrocyte sedimentation rate or C reactive protein. In the other children venesection was performed to investigate short stature (two cases), possible food allergy (one case), and asthma (three cases). All children were undergoing venesection for other investigations.

Chronic inflammatory bowel disease
All patients had been assigned a definite diagnosis of Crohn's disease or ulcerative colitis on the basis of radiological and histological findings and had undergone colonoscopy and barium followthrough investigation in the preceding 12 months. The children with Crohn's disease were divided into two groups on the basis of colonic involvement. Those with disease affecting the small bowel up to and including the terminal ileum, but with no evidence of macroscopic or microscopic involvement of the caecum or colon, were labelled as having small bowel Crohn's disease. Those whose disease included the caecum or colon, whether or not there was also small bowel involvement, were included in the large bowel Crohn's disease group.

TABLE I   Median (range) tumour necrosis factor α (TNFα) and disease indices (SEM) in relapse and remission of childhood inflammatory bowel disease

Condition	No	Erythrocyte sedimentation rate (mm in the 1st hour)	C reactive protein (mg/l)	TNFα (pg/ml)	p*
Control subjects	31			<10 (0–18)	
Small bowel Crohn's disease in remission	17	7·3 (1·97)	10·7 (2·18)	<10 (0–30)	NS
Small bowel Crohn's disease in relapse	13	27·38 (4·65)	40·1 (6·88)	<10 (0–30)	NS
Colonic Crohn's disease in remission	25	14·4 (1·88)	14·1 (2·30)	<10 (0–30)	NS
Colonic Crohn's disease in relapse	25	23·2 (2·72)	37·7 (4·49)	16 (0–60)	<0·00001
Ulcerative colitis in remission	23	13·47 (2·93)	10·7 (2·16)	<10 (0–18)	NS
Ulcerative colitis in relapse	11	36·2 (4·7)	26·1 (10·45)	27 (0–150)	<0·00001
Distal colitis in relapse	5	39·3 (14·1)	41·2 (20·2)	<10 (0–12)	NS

*Probability values, by Kolmogorov-Smirnov 2 group test, comparing disease group median serum TNFα with that of control subjects. NS=not significant.
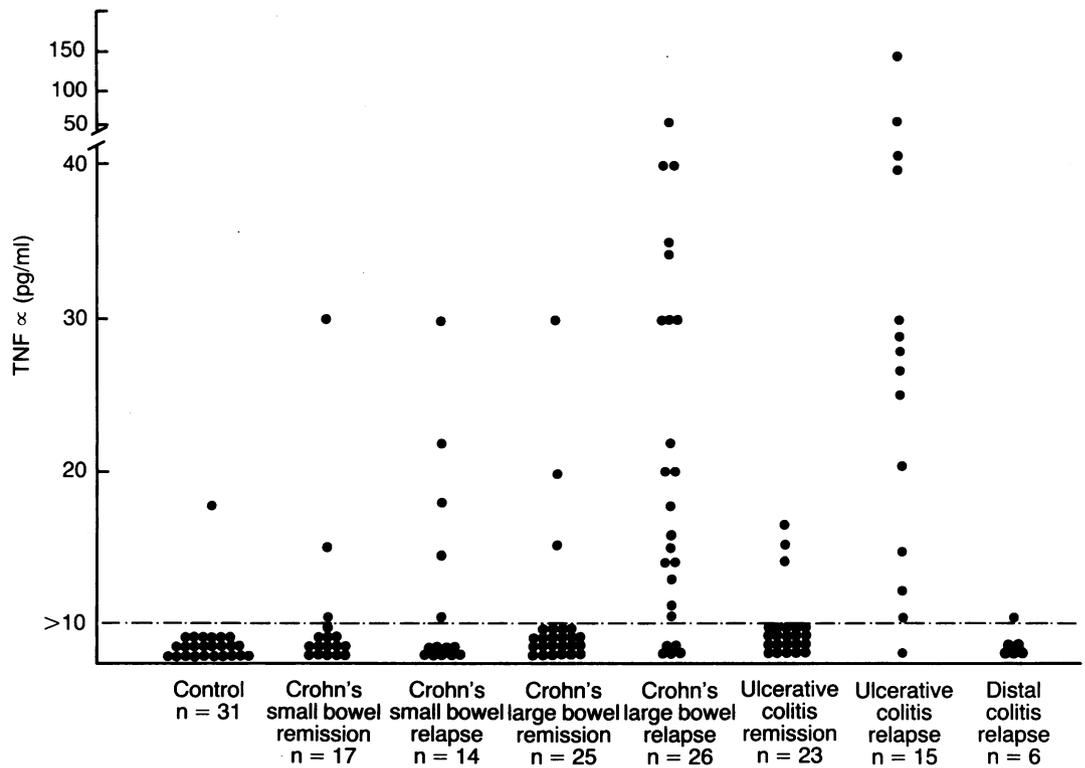
Serum TNFα (pg/ml) in control subjects and in disease groups.

DISEASE ACTIVITY

Patients were assessed in the paediatric inflammatory bowel disease clinic at two month intervals if well and every two to four weeks if not. Disease activity was assessed on the basis of reported symptoms, change in weight, clinical examination, and investigation, with the patient classified as follows. (a) Fully in remission (asymptomatic, appropriate weight gain, no abdominal tenderness or masses, no active perianal or oral disease, no appreciable rise in disease indices at last review). (b) Partially relapsed (minor episodic abdominal pain without systemic upset; active perianal disease; poor weight gain; isolated rise in disease indices at last review). (c) Relapsed (recurrent moderate or severe abdominal pain or diarrhoea >5 times daily with stools containing blood or mucus, or both; appreciable weight loss; abdominal tenderness or mass). Only those patients who were classified as fully relapsed or fully in remission were included in the study.

ASSESSMENT OF GROWTH AND PUBERTAL STAGE

All patients underwent formal auxological measurement and pubertal staging at each clinic visit and during any admission. Standing height was measured by the same observer (VAL) using a stadiometer and height velocity was calculated over the preceding three or six months. A pubertal stage was assigned on the basis of genital development in boys and breast development in girls using standard ratings from stage 1 (prepubertal) to stage 5 (adult).[12]

TNFα ASSAY PROTOCOL

After venesection blood was stored overnight at 4°C before being centrifuged at 2000 rpm for 10

minutes and the supernatant separated. The serum was then stored at $-70°C$ until assayed.

Aliquots of 50 µl of serum were assayed for TNFα by the enzyme linked immunoadsorbent assay (ELISA) technique, using a commercially available assay (T Cell Sciences, Cambridge MA, USA). Results were expressed in pg/ml, with the lower limit of detection of the assay being 10 pg/ml.

STATISTICAL ANALYSIS

The Kolmogorov-Smirnov 2 group test was used for comparison of TNFα and pubertal stages between groups and the unpaired Student's t test for analysis of erythrocyte sedimentation rate, C reactive protein, and height velocity.

Results

TNFα CONCENTRATION AND DISEASE ACTIVITY

Serum TNFα was significantly raised above control values (30 out of 31 below 10 pg/ml, range <10–18) in only two disease states, both associated with colonic inflammation (Figure and Table I): ulcerative colitis in relapse (n=11, median 27 pg/ml, range <10–150, $p<0.000007$) and Crohn's colitis in relapse (n=25, median 16 pg/ml, range <10–60, $p<0.000002$). Relapse of small bowel Crohn's disease alone, with no evidence of colonic involvement, was not associated with a significant rise in TNFα above control values (n=13, median <10 pg/ml, range <10–30, $p>0.2$), despite insignificant differences between the small and large bowel groups in the severity of relapse as measured by disease indices (see Table I: mean (SEM) erythrocyte sedimentation rate 27·38 (4·65) v 23·2 (2·72) mm in 1st hour, mean (SEM) C reactive protein 40·1

TABLE II   *Current age and mean (SEM) growth velocity in entire group and in patients in stage 1 puberty in relapse and remission groups*

| Condition | Age (years) | Growth velocity | | |
		Entire group (cm/year)	Patients in stage 1 puberty (cm/year)	p*
Control subjects	11·35 (0·51)			
Small bowel Crohn's disease in remission	13·74 (0·61)	6·49 (0·78)	6·48 (0·89, n=4)	NS
Small bowel Crohn's disease in relapse	14·48 (0·49)	5·24 (0·89)	3·78 (0·83, n=5)	
Colonic Crohn's disease in remission	13·34 (0·57)	4·90 (0·55)	4·71 (0·69, n=9)	NS
Colonic Crohn's disease in relapse	13·20 (0·34)	2·35 (0·47)	2·58 (0·71, n=11)	<0·001
Ulcerative colitis in remission	12·66 (0·74)	5·59 (0·48)	5·30 (0·18, n=10)	NS
Ulcerative colitis in relapse	11·58 (0·99)	2·14 (0·62)	2·50 (0·98, n=9)	<0·001
Distal colitis in relapse	12·4 (1·06)	5·64 (0·74)		NS

*Probability values, by unpaired Student's t test, comparing group mean height velocity (entire group) with that in subgroup with relapsed small bowel Crohn's disease. NS=not significant.

(6·9) v 37·7 (4·49) mg/l, p>0·2). In the small group with distal colitis alone serum TNFα was not raised above control values despite a greater rise in the sedimentation rate than in the group with relapsed colonic Crohn's disease (39·3 (14·1) v 23·2 (2·72), p<0·05) and a similar rise in C reactive protein. Ulcerative colitis in relapse was associated with a higher sedimentation rate than relapsed colonic Crohn's disease (36·2 (4·7) v 23·2 (2·72), p<0·007) with no significant difference in concentrations of C reactive protein or TNFα.

TNFα CONCENTRATION AND CURRENT HEIGHT VELOCITY

Current mean growth velocity was significantly lower in the groups with relapse of colonic Crohn's disease (p<0·001) and ulcerative colitis (p<0·001) than in the groups with the equivalent condition in remission (Table II). Although for the groups with small bowel Crohn's disease there was no significant difference between those in relapse and remission, analysis of the subgroups in stage 1 puberty showed significantly lower growth in those with symptomatic relapse (p<0·05).

Current growth velocity was significantly greater in the group with relapsed small bowel Crohn's disease than in those with relapsed Crohn's colitis (p<0·0002) or ulcerative colitis (p<0·0005).

No significant differences were found between the groups in overall pubertal staging, although patients in the group with small bowel Crohn's disease in relapse were significantly older (mean (SEM) age 14·48 (0·49) years) than those with colonic Crohn's disease (13·20 (0·34) years) or ulcerative colitis (11·58 (0·99) years) in relapse (p<0·05). Further analysis of the subgroups in stage 1 puberty, however, showed no significant differences between the groups, although the number of cases was small. The group with distal colitis had less impairment of growth than the groups with relapsed colonic Crohn's disease (p<0·02) or ulcerative colitis (p<0·0004).

Fifteen of the 48 patients with colonic disease in remission were taking prednisolone (5–30 mg)

daily compared with 27 of the 46 with colonic disease in relapse (7·5–40 mg), two of the 17 with small bowel disease in remission (5–30 mg), and three of the 13 with small bowel disease in relapse (5–20 mg). No significant differences in serum TNFα or height velocity were found between those taking prednisolone and those not taking corticosteroid treatment.

Discussion

These results show a clear difference between the concentrations of serum TNFα in children with relapsed colonic inflammatory bowel disease and those with relapsed Crohn's disease affecting the small bowel alone. The TNFα in serum is probably an overspill from that produced locally by activated macrophages in the colon and is therefore presumably an underestimate of local production. In support of this, a similar variation between local and systemic TNFα concentration has been shown by Nadal et al,[11] who found concentrations in the cerebrospinal fluid of 57–20 000 pg/ml in 11 children with bacterial meningitis, with serum TNFα detectable in only one.

Most TNFα is produced in activated macrophages and monocytes, although it is produced to some extent by hepatic Kupffer cells, cerebral astrocytes, and microglial cells.[1-4] Activated macrophages and T cells are abundant in intestinal mucosa in inflammatory bowel disease[13] and in recent experiments it has been shown that production of TNFα is increased at the single cell level,[14] thus showing formally that at least some of the TNFα detected in serum in inflammatory bowel disease is gut derived.

Populations of histiocytes and macrophages with different morphology and biochemical activity have been found in the small bowel compared with the colon.[15] It is thought that this difference reflects the requirement for antigen handling in the small intestine as opposed to scavenging in the bacteria laden colon. In colonic inflammatory bowel disease a further distinct group of macrophages has been found to replace those normally present in the colon, the population returning to normal with treatment.[15] Variation in expression of surface markers on T cells and macrophages between colonic and small bowel inflammatory bowel disease has more recently been shown.[16] These results suggest that our findings of increased serum TNFα in relapsed colonic but not small bowel disease may reflect fundamental differences between the populations of macrophages in these sites.

TNFα has been shown in animals to have a cytopathic effect on bowel mucosa,[17] probably mediated in part by platelet activating factor,[18] raising the possibility that it may be involved in the pathogenesis of inflammatory bowel disease. Multifocal gastrointestinal infarction, mediated by enhanced procoagulant activity, has in fact recently been proposed as a pathogenetic mechanism in Crohn's disease,[19] and raised concentrations of interleukin-1 have been reported.[20 21] Production of TNFα from activated macrophages, and its subsequent systemic action, has been attenuated by administration of non-steroidal anti-inflammatory drugs or

corticosteroids,[4][22] although we found no significant difference in serum TNFα between patients taking mesalazine, sulphasalazine, or prednisolone and those taking no drugs.

Suppression of linear growth is a common feature of childhood chronic inflammatory bowel disease, affecting up to 30% of patients.[23-27] Although children may present with growth failure alone, without symptoms or weight loss,[28] it is currently accepted that nutritional deficiency is the major cause of the growth failure and that caloric supplementation can restore growth.[23][25-27][29-32] Somatomedin C (insulin like growth factor 1) concentrations have been found to be low in growth impaired children with childhood inflammatory bowel disease and to increase as calorie intake improves.[33] Delayed gastric emptying, which may contribute to poor oral intake, has also been found in malnourished children with Crohn's disease.[34]

Although there seems to be major variations between the reported studies in the amount of caloric supplementation required to reinstitute growth and the nutritional supplementation was provided in several of the studies by methods which may in themselves be therapeutic,[35-37] such as parenteral nutrition[23][31][32] or elemental diet,[27] it is clear that restoring caloric intake, even using a polymeric non-elemental diet[29] or by aggressive oral nutrition[25] may lead to improvement of linear growth velocity in many patients.

The possible contribution of growth hormone deficiency to the growth suppression of chronic inflammatory bowel disease has been more controversial. After the report by McCaffery et al[26] of impaired growth hormone response to insulin induced hypoglycaemia in 11 of 14 growth retarded patients, subsequent studies have failed to show consistent abnormalities of growth hormone production.[38-40] These studies may be criticised on the grounds that growth hormone reserve was not retested in remission and that growth hormone concentrations in the normal range were considered appropriate, although raised plasma concentrations are normally found in protein-calorie malnutrition.[41] In the one report in which growth hormone reserve was retested it was found to be low in relapse and normal in remission.[42]

The value of surgical resection in stimulating growth, particularly in children in early puberty, has been emphasised by recent reports.[43-45] Such pronounced improvement of growth velocity, after resection of affected bowel, suggests that one or more mediators may be produced locally which have an inhibitory effect upon growth.

Although it is not possible to infer such direct inhibition from these results, TNFα could potentially affect growth by its adverse effects on appetite, nutrition, and growth hormone production. Its effects on nutrition are profound, with chronic production causing wasting despite unlimited intake.[46] In vitro studies have shown catabolic changes at the cellular level.[47][48] Administration of recombinant TNFα to animals and humans causes delayed gastric emptying,[49] profound anorexia, weight loss, and a redistribution of body proteins with depletion of skeletal muscle protein and increase in hepatic acute phase protein synthesis.[50][51] Hepatic expression of albumin mRNA was decreased by 90% after TNFα administration to mice.[52] In addition, TNFα has been shown in vitro to affect anterior pituitary hormone release,[53] specifically diminishing release of growth hormone.[54]

Many of the children in this study were passing through puberty, or alternatively suffering pubertal delay because of their disease. The most unusual finding was the relatively good growth of the group with relapsed small bowel Crohn's disease, and it seems likely that more members of this group were undergoing their pubertal growth spurt, despite the similarities in overall pubertal staging between the groups. The mean age of this group was significantly greater than the others and only this subgroup in stage 1 puberty had a lower mean height velocity than that of the entire group. Further study of growth and TNFα production in prepubertal children with chronic inflammatory bowel disease is required.

With the potential advent of therapeutic monoclonal anti-TNFα antibodies in conditions where TNFα may be playing a pathological part, it is clear that further work is required to determine the site of TNFα production in chronic inflammatory bowel disease and to establish whether its excess production does play a part in mucosal inflammation. In our study the highest serum TNFα (150 pg/ml) was found in a girl in whom toxic dilatation of the colon was considered possible (she settled on conservative management) and it will be important to study TNFα production in cases where this diagnosis is certain. Should TNFα be found to have a role in the pathogenesis of this life threatening complication (and its credentials for direct bowel wall destruction are well established from animal work),[17][18] then therapeutic monoclonal anti-TNFα antibodies may present an important alternative to the radical surgery currently required. Their possible future use in less serious relapse of disease will be more difficult to assess, and it is important that those looking after children with chronic inflammatory bowel disease should aim for precise localisation of the affected bowel and stringent monitoring of growth.

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