Secretion imbalance between tumour necrosis factor and its inhibitor in inflammatory bowel disease

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Abstract

Background—Tumour necrosis factor (TNF) α and TNF- β are soluble ligands binding to TNF receptors with similar activities; soluble TNF receptors neutralise TNF activity by acting as inhibitors. Little is known about the cytokine/soluble receptor role in inflammatory bowel disease (IBD).

Aims—To test the hypothesis that an imbalance in secretion between TNF and TNF inhibitors plays a role in gut inflammation in patients with IBD.

Methods—The secretion of TNF- α , TNF- β , and soluble TNF receptors was compared in the culture supernatants of colonic biopsy specimens and isolated lamina propria mononuclear cells from patients with active colonic IBD.

Results—Spontaneous secretion of TNF- α in involved IBD mucosa was higher than in normal control and self limited colitis mucosa. Secretion of TNF- β was higher in patients with Crohn's disease than in those with ulcerative colitis. Soluble TNF receptor in IBD mucosa inhibited TNF activity. Type 2 soluble receptor release from IBD mucosa was increased in active inflammation; release from lamina propria cells was not increased. Mucosal TNF- α production correlated with severity of disease.

Conclusions—Results showed enhanced secretion of TNF- α but failure to release enhanced amounts of soluble TNF receptor in lamina propria mononuclear cells of patients with IBD. An imbalance in secretion between TNF and TNF inhibitor may be implicated in the pathogenesis of IBD. (Gut 1998;43:203-209)

Keywords: Crohn's disease; inflammation; mucosal immunology; soluble TNF receptor; tumour necrosis factor; ulcerative colitis

matory and anti-inflammatory cytokine production.¹ Tumour necrosis factor (TNF) ligands and the natural inhibitor of TNF, soluble TNF receptor (sTNF-R), play a major role in the regulation of inflammatory diseases such as sepsis and rheumatoid arthritis.^{2,3} The gastrointestinal tract is a prime target of TNF produced by blood mononuclear cells, accumulating 10% of a systemically administered dose.⁴ Administration of TNF induces extensive bowel haemorrhage and necrosis.⁵ TNF- α and TNF- β (lymphotoxin α) are soluble ligands binding to TNF receptors with similar effects; sTNF-R neutralises TNF activity.⁶ TNF mediated apoptosis is evoked by the intracellular death domain of the TNF receptors in cells.⁷ The two soluble TNF receptors, p55 type 1 (sTNF-R1) and p75 type 2 (sTNF-R2), are derived from the extracellular domains of TNF receptors by enzymatic cleavage.

Recent reports suggest that antibodies against TNF- α are therapeutic in Crohn's disease.⁸⁻¹⁰ Serum and stool samples from patients with active Crohn's disease or ulcerative colitis contained increased concentrations of TNF- α , probably derived from inflammatory cells in the gut lesions.^{11,12} However, others have reported that serum TNF- α levels and mucosal RNA transcript levels were not elevated in patients with IBD.^{13,14} Although TNF has been studied in IBD, little is known about the cytokine/soluble receptor role in the pathogenesis of IBD. The aim of this study was to test the hypothesis that an imbalance in secretion between TNF and TNF inhibitors plays an important role in gut inflammation, specifically in patients with IBD. We examined the secretion of TNF- α , TNF- β , and soluble TNF receptors in the culture supernatants of both colonic biopsy specimens and isolated lamina propria mononuclear cells (LPMCs) from patients with Crohn's disease, ulcerative colitis, and controls.

Patients and methods

ORGAN CULTURE

Seventy patients with IBD, being treated in the Hospital of Tohoku University School of Medicine, were prospectively included in the study. All patients had active disease, as assessed by colonoscopy. The diagnosis was based on routine clinical, radiological, and endoscopic criteria and confirmed by histological evaluation. Table 1 presents details of the patients with IBD. The activity of Crohn's disease was assessed according to the criteria of Van Hees *et al.*¹⁵ Index values of 100-150 can

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Table 1 Characteristics of patients in the tissue culture study

	Crohn's disease	Ulcerative colitis
No of patients	35	35
Mean (range) age (y)	24.4 (17–59)	28.5 (11–63)
Men/women	28/7	26/9
Mean (range) disease duration (y)	1.1 (0.3–8)	1.8 (0.3–5)
Disease activity (n)		
Severe	4	8
Moderate	12	10
Mild	19	17
Bowel movement (n)		
Ileocolonic disease	21	0
Colonic disease	14	16
Left side colon alone	0	19
Current treatment		
Steroids >20 mg/day	0	6
Sulphasalazine plus steroids	5	8
Mean (range) length of steroid therapy (months)	5.8 (3.7–8.0)	2.0 (0.2–6.0)
TPN/elemental diet	9	0
Sulphasalazine	10	13
No medication	11	8

Disease activity assessments were based on the Van Hees index for Crohn's disease and the Truelove and Witts index for ulcerative colitis.

TPN, total parenteral nutrition.

Table 2 Characteristics of patients in the isolated cell culture study

	Crohn's disease	Ulcerative colitis
No of patients	16	18
Mean (range) age (y)	27 (15–45)	29 (17–56)
Men/women	12/4	9/9
Mean (range) disease duration (y)	2.2 (0.3–5)	2.6 (0.2–8)
Disease activity (n)		
Severe	2	6
Moderate	4	9
Mild	10	3
Bowel movement (n)		
Ileocolonic disease	12	0
Colonic disease	4	16
Left side colon alone	0	2
Current treatment		
Steroids >20 mg/day	0	10
Sulphasalazine plus steroids	3	8
Mean (range) length of steroid therapy (months)	2.4 (1–3.8)	4.1 (1–18)
TPN/elemental diet	13	0
Sulphasalazine		
No medication		

Disease activity assessments were based on the Van Hees index for Crohn's disease and the Truelove and Witts index for ulcerative colitis.

TPN, total parenteral nutrition.

be regarded as indicating mild inflammatory activity, values of 150–210 as indicating moderate activity, and values above 210 as indicating severe activity. The severity of ulcerative colitis was assessed according to the classification of Truelove and Witts.¹⁶

Normal control tissue samples were obtained from 14 patients with colonic polyps (four women; mean age 43 years, range 35–58). Inflammatory control tissue samples were obtained from eight patients with self limited colitis (three women; mean age 38 years, range 24–45).

Biopsy specimens were obtained by colonoscopy under direct vision from involved and normal appearing colonic sites in patients with Crohn's disease and ulcerative colitis, and from normal mucosa of patients with a colonic polyp. Colonic mucosal explants were cultured for 24 hours at 37°C with 95% O₂ and 5% CO₂ by the established procedure.^{17,18} All culture media consisted of RPMI 1640 medium and 10% fetal calf serum (Life Technologies, Great Island, USA) with penicillin (100 U/ml) and streptomycin (100 µg/ml). After culture for 24 hours, the organ culture medium was collected

and used for TNF- α , TNF- β , and sTNF-R assays. In our data, biopsy specimen wet weight was shown to correlate closely with protein content of tissue homogenates.

LAMINA PROPRIA MONONUCLEAR CELL CULTURE

Thirty four patients with IBD admitted for therapeutic bowel resection were studied. Table 2 shows the clinical data and treatment at the time of the study. Disease severity was higher in the ulcerative colitis group than in the Crohn's disease group. The surgical control group consisted of 10 patients (two women; aged 38–65 years, mean age 52), admitted for bowel resection for adenocarcinoma of the large bowel.

Isolation of lamina propria mononuclear cells (LPMCs) was performed as described previously.^{18,19} The LPMCs were purified by density gradient centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden). The LPMCs were always 95–98% viable as determined by trypan blue staining. These cells were incubated in 24 well cell culture plates for 24 hours at 37°C in 95% air and 5% CO₂ with and without 10 µg/ml of *Escherichia coli* lipopolysaccharide (LPS; 0128 *E. coli*, Sigma, St Louis, USA) as enteric bacterial cell wall components. The supernatant was then collected and used for TNF- α , TNF- β , and sTNF-R assays.

The leucocyte subpopulations present within the isolated LPMCs were analysed by flow cytometry using monoclonal antibodies that recognise CD4, CD8, CD3, and CD11b. No significant differences in the percentages of cell subpopulations were detected, when either involved or non-involved LPMCs from ulcerative colitis mucosa or Crohn's disease mucosa were compared with controls. These findings are consistent with previous observations.²⁰

IMMUNOASSAYS FOR TNF LIGANDS AND SOLUBLE RECEPTORS

TNF- α levels in the media were measured using a human TNF- α immunoenzymetric assay kit (Medgenix, Fleurus, Belgium). TNF- β levels in supernatant were measured using a human TNF- β enzyme linked immunosorbent assay kit (R&D systems, Minneapolis, USA). The limit of sensitivity of the assays used was 4.4 pg/ml for TNF- α , and 7.0 pg/ml for TNF- β . Anti-TNF- α antibody and anti-TNF- β antibody did not cross react with any other cytokine. Soluble TNF receptor levels in the supernatant were measured using a human soluble TNF receptor type 1 (p55) and type 2 (p75) immunoenzymetric assay kit (Medgenix). The limit of sensitivity of the assays is 1.0 pg/ml for sTNF-R1, and 1.0 pg/ml for sTNF-R2. All samples were analysed in duplicate. Results of cytokine concentrations were expressed according to the weight of the biopsy specimen (pg/mg tissue/ml culture medium) in supernatant of organ culture and according to the number of isolated cells (pg/10⁶ cells/ml culture medium) in the supernatant fluid of mononuclear cell culture.

Table 3 Release of TNF and inhibitors in the tissue culture study

Measured cytokine	Normal controls (n=14)	Inflammatory controls (n=8)	Involved mucosa		Non-involved mucosa	
			CD (n=35)	UC (n=35)	CD (n=30)	UC (n=22)
TNF- α	5.6 (4.6–8.4)	16.9 (9.0–30.8)	49.2 (25–100)*†	42.5 (24.0–79.2)*†	18.7 (9.4–25.8)*	3.8 (2.1–13.0)
TNF- β	1.4 (1.06–1.8)	1.94 (1.4–2.38)	2.78 (1.65–3.78)*	1.5 (1.06–1.64)	1.5 (1.4–2.9)	1.5 (1.3–2.3)
sTNF-R1	100 (62–130)	124 (112–138)	118 (72–191)	71 (53–101)	140 (85–148)	148 (132–178)
sTNF-R2	58 (32–72)	88 (82–95)	208 (158–335)*	205 (120–272)*	58 (55–120)	53 (35–92)

Cytokine levels and soluble receptors levels are given as pg/mg tissue wet weight, and all data are presented as median (interquartile range).

*p<0.01 v normal controls; †p<0.01 v inflammatory controls.

UC, ulcerative colitis; CD, Crohn's disease.

Table 4 Release of TNF and inhibitors in the isolated cell culture study

Measured cytokine	Normal controls LPMCs (n=10)	Involved LPMCs		Non-involved LPMCs	
		CD (n=16)	UC (n=18)	CD (n=16)	UC (n=10)
TNF- α	21.5 (15–79)	385 (95–745)*†	725 (158–1278)*†	64.0 (38–74)	48.0 (20–127)
TNF- β	25.0 (18–26)	20.5 (16–24)	32.0 (22–41)	30.5 (25–32)	30.0 (28–34)
sTNF-R1	533 (450–578)	475 (245–700)	450 (220–600)	550 (425–590)	500 (208–320)
sTNF-R2	200 (180–410)	545 (345–1525)	500 (200–1300)	750 (340–1520)	250 (200–320)

Cytokine levels and soluble receptors levels are given as pg/mg tissue wet weight, and all data are presented as median (interquartile range) adjusted to 100 000 cells/well.

*p<0.01 v controls; †p<0.01 v non-involved LPMCs.

UC, ulcerative colitis; CD, Crohn's disease.

INHIBITION OF TNF ACTIVITY IN CYTOTOXIC ASSAY

Biological TNF activity was measured by the WEHI cell killing assay.²¹ The inhibitory activity of the culture media was assessed by its ability to inhibit TNF- α in the WEHI 164 cytotoxic assay. Briefly, mouse WEHI-164 cells (obtained from Tohoku University Cancer Cell Collection) were seeded in 96 well microplates in triplicate at a density of 20 000 cells/well. Culture samples (100 μ l) from five patients with IBD were added alone or with the addition of 10 μ l of monoclonal antihuman p75 TNF-R2 antibody (5 μ g/ml; Genzyme, Cambridge, USA) or p75 sTNF-R2 (2 ng/ml; Medgenix). Culture medium (100 μ l) and actinomycin D (1 μ g/ml; Wako) was then added after five hours. Monoclonal antihuman p75 TNF-R2 antibody binds specifically to the extracellular domain of human TNF-R2 and recognises soluble forms of TNF-R2. Cell viability was assessed 24 hours later at 37°C and

5% CO₂ by tetrazolium salts colorimetric assay (Boehringer Mannheim, Mannheim, Germany).

STATISTICS

Results are expressed as median (interquartile range, IQR). The results were compared by the non-parametric Kruskal-Wallis test and the Pearson *r* correlation test. Significance was accepted as p<0.05 and determined by the Bonferroni (Dunn) post hoc test.

ETHICS

The acquisition and use of human blood and tissue for these studies were approved by the Human Ethics Committee of Tohoku University School of Medicine.

Results

RELEASE OF TNF- α AND TNF- β BY ORGAN CULTURE

Spontaneous release of TNF- α measured in the supernatant fluid of involved Crohn's colitis mucosa and involved ulcerative colitis mucosa was significantly higher than in normal control mucosa or inflammatory control mucosa (table 3). Non-involved mucosa from patients with Crohn's disease produced significantly higher amounts of TNF- α than did that from normal controls. Concentrations of TNF- β measured in the medium of involved mucosa from patients with Crohn's disease were significantly higher than in that from involved mucosa and non-involved mucosa from patients with ulcerative colitis or normal controls.

RELEASE OF SOLUBLE TNF RECEPTOR BY ORGAN CULTURE

Spontaneous release of sTNF-R2 measured in the supernatant fluid of involved Crohn's colitis mucosa and involved ulcerative colitis mucosa was significantly higher than from normal control mucosa or inflammatory control

Figure 1 Secretion ratio of TNF- α :sTNF-R2 in organ culture. CD, Crohn's disease; UC, ulcerative colitis.

Figure 2 Secretion ratio of median TNF- α :median sTNF-R2 in LPMCs cultured with LPS. CD, Crohn's disease; UC, ulcerative colitis.

Table 5 Relation of disease severity and TNF system in isolated mucosal cells

	Mild	Moderate	Severe	r	p Value
TNF- α	72.1 (38–82)	498 (330–1280)	1210 (900–1970)	0.739	<0.0001
TNF- β	30.6 (21–40)	30.0 (22.5–37.5)	19.5 (14.8–24.0)	-0.410	0.218
sTNF-R1	500 (200–560)	400 (162–475)	500 (425–600)	-0.362	0.129
sTNF-R2	375 (200–800)	455 (290–1100)	1060 (200–1800)	0.262	0.089

LPS stimulated production (pg/ml 10^6 cells). Values are median (interquartile range).

mucosa ($p < 0.001$; table 3). Secretion of sTNF-R2 tended to correlate with the amount of TNF- α released in mucosa from IBD patients ($r = 0.719$, $p < 0.001$).

When the ratio TNF- α :sTNF-R2 was calculated, an increased production ratio was not observed with involved mucosa of patients with IBD compared with ratios from non-involved mucosa of patients with IBD, control mucosa, and infectious colitis mucosa (fig 1).

In the organ culture, sTNF-R1 secretion was no higher in patients with IBD than in controls. No correlation was observed between levels of TNF- α and levels of sTNF-R1 ($r = 0.32$, NS).

Figure 3 Inhibitory activity of TNF by soluble TNF receptor in supernatant (S) of organ culture and LPMC culture, reversible cytotoxicity by monoclonal antibody to p75sTNF-R2 (R2Ab), and inhibition by recombinant human soluble TNF-R2 (sR2). Culture samples from five patients with IBD were examined in triplicate cultures. Results expressed as median (IQR). * $p < 0.05$ versus S group, R2Ab plus sR2 group.

RELEASE OF TNF- α AND TNF- β BY ISOLATED CELL CULTURES

Under LPS stimulated conditions, secretion of TNF- α in the supernatant fluid from isolated LPMCs of involved mucosa from Crohn's disease and ulcerative colitis was significantly higher than from control mucosa or non-involved mucosa from Crohn's disease and ulcerative colitis (table 4).

Spontaneous TNF- α secretion by isolated LPMCs of involved mucosa from Crohn's colitis (median 270 (IQR 80–1131) pg/ml) and ulcerative colitis (275 (90–484) pg/ml) was also higher than that produced by non-involved mucosa from Crohn's disease (30.0 (18–205) pg/ml) and ulcerative colitis (36.0 (31–57.3) pg/ml) or that of control mucosa (26.0 (18–54.3) pg/ml) ($p < 0.05$). Under LPS stimulated conditions, TNF- β secretion was not higher in LPMCs from patients with IBD than from controls (data not shown).

RELEASE OF SOLUBLE TNF RECEPTORS BY ISOLATED CELL CULTURES

Release rates of sTNF-R2 by LPS stimulated LPMCs of the involved mucosa from Crohn's colitis and ulcerative colitis were not significantly higher than those of non-involved mucosa from Crohn's disease and ulcerative colitis and control mucosa (table 4). No correlation was observed between levels of TNF- α and levels of sTNF-R2 ($r = 0.18$, NS). Spontaneous sTNF-R2 secretion by isolated LPMCs of involved mucosa from Crohn's colitis (median 240 (IQR 170–390) pg/ml) and ulcerative colitis (680 (340–1100) pg/ml) was no higher than that produced by non-involved mucosa from Crohn's disease (260 (210–360) pg/ml) and ulcerative colitis (205 (170–350) pg/ml) or that of control mucosa (940 (780–1100) pg/ml) ($p < 0.05$).

When the ratio of median TNF- α :median sTNF-R2 was calculated, an increased production ratio was observed in LPMCs from involved mucosa of patients with Crohn's disease and ulcerative colitis (fig 2). In LPS stimulated LPMCs, sTNF-R1 secretion was no higher in patients with IBD than in controls.

RELATION OF MUCOSAL TNF SYSTEM AND SEVERITY OF DISEASE

Clinical disease severity tended to correlate with the secretion of TNF- α in colonic lesions ($r = 0.581$, $p < 0.0001$) and in LPS stimulated LPMCs from patients with IBD ($r = 0.739$, $p < 0.0001$; table 5). No correlation was observed between severity and the levels of TNF- β , sTNF-R1, or sTNF-R2.

EFFECT OF SOLUBLE TNF RECEPTOR ON TNF ACTIVITY

To assess the function of sTNF-R, we incubated culture samples with neutralising concentrations of monoclonal antibody to sTNF-R. TNF cytotoxicity was significantly higher in organ culture samples with sTNF-R2 monoclonal antibody than in samples only (53.6 (46.0–70.5)% versus 26.7 (22.0–34.8)%, $p < 0.05$; fig 3). Furthermore, the effect of inhibitor could be reversed by incubation

with human sTNF-R2 (29.7 (4.5–36.0)%). No cytotoxicity to WEHI 164 cells was observed after treatment with monoclonal antibody alone.

Discussion

Our study showed that a large amount of TNF- α was released by LPMCs from inflamed mucosa of patients with IBD. Breese *et al* also reported that TNF- α positive cells increased in the mucosa of inflamed intestine.²² When TNF mRNA has been measured, different results have been obtained. Isaacs *et al* could not detect any TNF- α transcripts in the mucosal samples from patients with IBD.¹⁴ It is now known however that most autocrine cytokines and soluble cytokine receptors are derived from the cell surface by enzymatic cleavage; messenger RNA analysis for cytokine and the receptor is therefore not informative, as it does not discriminate between the cell surface form and the soluble form.²³ Cytokine membrane molecules are enzymatically cleaved from the cell surface and then released into the extracellular medium in the form of soluble fragments.²⁴ In particular, TNF- α , a proinflammatory membrane anchored cytokine, is released from the membrane glycoprotein of metalloprotease disintegrins by TNF- α convertase.²⁵ Several physiological processes are known to regulate the activity of TNF- α with respect to synthesis, processing, and effects on the target cell.²⁶ Therefore, this study of soluble TNF and soluble TNF inhibitor may be important for determination of the pathogenesis of IBD.

Our study showed that LPS stimulation increased TNF- α secretion only twofold in LPMCs of non-involved mucosa and mucosa from controls. Similar results were reported by Reinecker *et al*.²⁰ The relatively poor response of LPMCs to LPS indicates previous stimulation or a state of relative anergy.²⁷ Our study showed that TNF- α production in LPMCs of severe inflamed mucosa could be enhanced further by LPS stimulation. We suggest that in newly recruited LPMCs, TNF- α production is enhanced by LPS stimulation, as Rugtveit *et al* report.²⁸

Our bioassay data showed that sTNF-R had a functional effect as a TNF inhibitor in inflamed mucosa. Spontaneous secretion of sTNF-R2 from inflamed mucosa in IBD was significantly higher than in control mucosa, suggesting that sTNF-R2 acts as a naturally occurring damper of the inflammatory process in IBD. LPS (endotoxin), a major component of the outer membrane of Gram negative bacteria, has a number of immunological activities. A release of sTNF-R2, but not sTNF-R1, is enhanced after activation with *E coli* LPS.²⁹ When stimulated by LPS, sTNF-R can induce TNF tolerance, a well controlled active response orchestrated to prevent excessive inflammation.^{2, 29} The LPS stimulated sTNF-R2 secretion by LPMCs obtained from patients with IBD was low. Rugtveit *et al* also did not observe any significant increase in the low levels of sTNF-R2 produced by LPMCs after any type of stimulation.²⁸ Foley *et al*

reported a decrease in TNF inhibitor in serum of patients with Crohn's disease compared with those with sarcoidosis or tuberculosis.³⁰ Bouma *et al* reported that TNF activity was decreased by bioassay in stimulated PBMCs of patients with ulcerative colitis.³¹ Different quantitative results can be obtained when TNF is measured in biological fluids by bioassay and immunoassay. Our TNF inhibitor bioassay showed the presence of the antigenic form of TNF that cannot be detected by routine bioassay, such as complexes with the soluble receptors. These results suggested that release of the TNF inhibitor from intestinal mononuclear cells is down regulated in IBD. Enteric bacterial derived LPS may initiate a sequence of inflammatory processes that are not appropriately down regulated by TNF inhibitor in IBD. LPS stimulated sTNF-R2 production is inhibited by bactericidal/permeability inducing protein.²⁹ Monajemi *et al* reported that mucosal levels of bactericidal/permeability inducing protein are increased in IBD.³² Soluble TNF receptors may regulate TNF activity; conversely, at a high TNF:sTNF-R ratio, these can act as carriers for TNF and prolong their activities by stabilising the trimeric structure.²⁹ The LPMCs in IBD lesions in particular had a high TNF:sTNF-R ratio. The intestinal mucosal mononuclear cells in IBD lesions may abrogate the function of TNF inhibitor.

TNF- β plays an important role for gut homing in intestinal lymphocytes and development in intestinal lymphoid organs.^{33, 34} Our results showed that the spontaneous production of TNF- β in the mucosa of patients with Crohn's disease was increased, but this was not observed in that of patients with ulcerative colitis. Bouma *et al* reported TNF- β production was increased in stimulated peripheral blood mononuclear cells of patients with Crohn's disease.³¹ We found that the normal appearing mucosa in patients with active Crohn's disease produced larger amounts of TNF- α than normal control mucosa; this was not observed in patients with ulcerative colitis. Reimund *et al* reported increased production of TNF- α by normal intestinal biopsy specimens from patients with Crohn's disease.³⁵ These findings suggest that Crohn's disease and ulcerative colitis have a different pathogenesis. TNF affects the tight junction between epithelial cells and increases the flow of solute between cells and across the epithelium.^{36, 37} Increased intestinal permeability is a consistent finding in patients with active Crohn's disease.³⁸ These studies are important and the sequence of appearances is shown in the neo-terminal ileum, where recurrent lesions progress from normal appearing mucosa of Crohn's disease by an effect of faecal stream.³⁹

It has been reported that IBD clinical activity is virtually independent of the severity of the mucosal lesions and tissue destruction.^{40, 41} Our study showed that production of TNF- α in the mucosa and the LPMCs was associated with severity of IBD; TNF- α may have a pivotal role in the degree of severity produced. We suggest that a relative deficiency of TNF inhibitor in involved intestinal mononuclear cells may con-

tribute to inflammation, which is relevant to current concepts of immunopathogenesis for IBD. Monoclonal anti-TNF- α antibody treatment was effective for patients with severe graft versus host disease and the gut lesions responded very well.⁴² Binding of anti-TNF- α antibody resulted in killing of TNF positive cells by antibody dependent cellular toxicity.⁴³ Therefore, it is more likely that anti-TNF induces tolerance in IBD, killing the TNF dysregulatory cells rather than simply neutralising TNF released from these cells.

A recombinant human sTNF-R2-Fc fusion protein has been developed for therapeutic neutralisation of TNF- α in patients with active rheumatoid arthritis.⁴⁴ We suggest that inhibition of TNF- α may have potential in the treatment of IBD. For example, inhibition of TNF- α by thalidomide was effective in a patient with Crohn's disease.⁴⁵ TNF is known to have affinity for heparin and an increase in the amount of sTNF-R shed is caused by heparin.⁴⁶ Heparin can be protective against the harmful effects of TNF and has been shown to be useful in the treatment of ulcerative colitis.⁴⁷ Anti-CD4 treatment also results in increased shedding of sTNF-R in patients with Crohn's disease.⁴⁸ Further investigation of the inhibitor, such as sTNF-R in autoimmune tissue damage, will allow us to decide the validity of our suggestion.⁴⁹⁻⁵⁰

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- 1 Sartor RB. Microbial factors in the pathogenesis of Crohn's disease, ulcerative colitis, and experimental intestinal inflammation. In: Kirsner JB, Shorter RG, eds. *Inflammatory bowel disease*. 4th edn. Baltimore: Williams & Wilkins, 1995:96-124.
- 2 Van Zee KJ, Kohn T, Fischer E, et al. Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor α in vitro and in vivo. *Proc Natl Acad Sci USA* 1992;**89**:4845-9.
- 3 Cope AP, Aderka D, Doherty M, et al. Increased levels of soluble tumor necrosis factor receptors in the sera and synovial fluid of patients with rheumatic diseases. *Arthritis Rheum* 1992;**3**(suppl):1160-9.
- 4 Beutler B, Milsark IW, Cerami A. Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. *J Immunol* 1985;**133**(suppl):3972-7.
- 5 Torimoto K, Sato N, Okubo M, et al. Development of multiple necrotizing enteritis induced by a tumor necrosis factor-like cytokine from lipopolysaccharide-stimulated peritoneal macrophages in rats. *Am J Pathol* 1990;**137**:1103-11.
- 6 Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996;**334**:1717-25.
- 7 Nagata S. Apoptosis by death factor. *Cell* 1997;**88**:355-65.
- 8 Van Dulleken HM, van Deventer SHJ, Hommes DW, et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology* 1995;**109**:129-35.
- 9 Stack WA, Mann SD, Roy AJ, et al. Randomised controlled trial of CDP571 antibody to tumour necrosis factor- α in Crohn's disease. *Lancet* 1997;**349**:521-4.
- 10 Targan SR, Hanauer SB, Van Deventer SHJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor α for Crohn's disease. *N Engl J Med* 1997;**337**:1029-35.
- 11 Braegger CP, Nicholls S, Murch SH, et al. Tumor necrosis factor alpha in stool as a marker of intestinal inflammation. *Lancet* 1992;**339**:89-91.
- 12 Murch SH, Lamkin VA, Savage MO, et al. Serum concentrations of tumor necrosis factor alpha in childhood chronic inflammatory bowel disease. *Gut* 1991;**32**:913-17.
- 13 Hyams JS, Treem WR, Eddy E, et al. Tumor necrosis factor-alpha is not elevated in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1991;**12**:233-6.
- 14 Isaacs KL, Sartor B, Haskill S. Cytokine messenger RNA profiles in inflammatory bowel disease mucosa detected by polymerase chain reaction amplification. *Gastroenterology* 1992;**103**:1587-95.
- 15 Van Hees PA, Van Elteren PH, Van Lier JH, et al. An index of inflammatory activity in patients with Crohn's disease. *Gut* 1980;**21**:279-86.
- 16 Truelove SC, Witts LJ. Cortisone in ulcerative colitis: a final report on a therapeutic trial. *BMJ* 1955;**2**:1041-4.
- 17 Eastwood GL, Trier TS. Organ culture of human rectal mucosa. *Gastroenterology* 1973;**64**:375-82.
- 18 Bull DM, Bookman MA. Isolation and functional characterization of human intestinal mucosal lymphoid cells. *J Clin Invest* 1977;**59**:966-74.
- 19 Noguchi M, Hiwatashi N, Liu Z, et al. Enhanced interferon- γ production and B7-2 expression in isolated intestinal mononuclear cells from patients with Crohn's disease. *J Gastroenterol* 1995;**30**(suppl 8):52-5.
- 20 Reinecker HC, Steffen M, Witthoef T, et al. Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1 β by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. *Clin Exp Immunol* 1993;**94**:174-81.
- 21 Espevik T, Nissen-Meyer J. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J Immunol Methods* 1986;**95**:99-105.
- 22 Breese EJ, Michie CA, Nicholls SW, et al. Tumor necrosis factor α -producing cells in the intestinal mucosa of children with inflammatory bowel disease. *Gastroenterology* 1994;**106**:1455-66.
- 23 Kaplan D. Autocrine secretion and the physiological concentration of cytokines. *Immunol Today* 1996;**17**:303-4.
- 24 Bazil V. Physiological enzymatic cleavage of leukocyte membrane molecules. *Immunol Today* 1995;**16**:135-40.
- 25 Black R, Rauch CT, Kozlosky CJ, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor- α . *Nature* 1997;**385**:729-33.
- 26 Eigler A, Sinha B, Hartmann G, et al. Taming TNF: strategies to restrain this proinflammatory cytokine. *Immunol Today* 1997;**18**:487-91.
- 27 Takasuka N, Tokunaga T, Akagawa KS. Preexposure of macrophages to low doses of lipopolysaccharide inhibits the expression of tumor necrosis factor- α mRNA but not IL-1 β mRNA. *J Immunol* 1991;**146**:3824-30.
- 28 Rugtveit J, Nilsen EM, Bakka A, et al. Cytokine profiles differ in newly recruited and resident subsets of mucosal macrophages from inflammatory bowel disease. *Gastroenterology* 1997;**112**:1493-505.
- 29 Leeuwenberg JFM, Dentener MA, Buurman WA. Lipopolysaccharide LPS-mediated soluble TNF receptor release and TNF receptor expression by monocytes. *J Immunol* 1994;**152**:5070-6.
- 30 Foley N, Lambert C, McNicol M, et al. An inhibitor of the toxicity of tumour necrosis factor in the serum of patients with sarcoidosis, tuberculosis and Crohn's disease. *Clin Exp Immunol* 1990;**80**:395-9.
- 31 Bouma G, Pool MO, Scharenberg JGM, et al. Differences in the intrinsic capacity of peripheral blood mononuclear cells to produce tumor necrosis factor alpha and beta in patients with inflammatory bowel disease and healthy controls. *Scand J Gastroenterol* 1995;**30**:1095-100.
- 32 Monajemi H, Meenan J, Lamping R, et al. Inflammatory bowel disease is associated with increased mucosal levels of bactericidal/permeability-increasing protein. *Gastroenterology* 1996;**110**:733-9.
- 33 Ni J, Porter AG, Hollander D. β 7 integrins and other cell adhesion molecules are differentially expressed and modulated by TNF- β in different lymphocyte populations. *Cell Immunol* 1995;**161**:166-72.
- 34 Banks TA, Rouse BT, Kerley MK, et al. Lymphotoxin- α -deficient mice. *J Immunol* 1995;**155**:1685-93.
- 35 Reimund JM, Wittersheim C, Dumont S, et al. Increased production of tumour necrosis factor- α , interleukin-1 β , and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. *Gut* 1996;**39**:684-9.
- 36 Mullin JM, Snock KV. Effect of tumor necrosis factor on epithelial tight junctions and transepithelial permeability. *Cancer Res* 1990;**50**:2172-6.
- 37 Mahraoui L, Heyman M, Plique O, et al. Apical effect of diosmectite on damage to the intestinal barrier induced by tumour necrosis factor- α . *Gut* 1997;**40**:339-43.
- 38 Wyatt J, Vogelsang H, Hubl W, et al. Intestinal permeability and the prediction of relapse in Crohn's disease. *Lancet* 1993;**341**:1437-9.
- 39 Rutgeerts P, Goobes K, Peeters M, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet* 1991;**338**:771-4.
- 40 Ghosh S, Ferguson A. Mucosal immunology research design should address not just cytokines and cells, but also clinical data and controls. *Clin Exp Immunol* 1994;**96**:377-8.
- 41 Cellier C, Sahmond T, Froguel E, et al. Correlations between clinical activity, endoscopic severity, and biological parameters in colonic or ileocolonic Crohn's disease. A prospective multicentre study of 121 cases. *Gut* 1994;**35**:231-5.
- 42 Herve BP, Flesch M, Tierghien P, et al. Phase I-II trial of a monoclonal anti-tumor necrosis factor α antibody for the treatment of refractory severe acute graft-versus-host disease. *Blood* 1992;**79**:3362-8.
- 43 Scallon BJ, Moore MA, Trinh H, et al. Chimeric anti-TNF- α monoclonal antibody cA2 binds recombinant transmembrane TNF- α and activates immune effector functions. *Cytokine* 1995;**7**:251-9.
- 44 Moreland LW, Baumgartner SW, Schiff MH, et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N Engl J Med* 1997;**337**:141-7.
- 45 Wettstein AR, Meagher AP. Thalidomide in Crohn's disease. *Lancet* 1997;**350**:1445-6.

- 46 Lanz M, Thysell H, Nilsson E, Olsson I. On the binding of tumor necrosis factor to heparin and the release in vivo of the TNF-binding protein I by heparin. *J Clin Invest* 1991; **88**:2026–31.
- 47 Gaffney PR, O'Leary JJ, Doyle CT, *et al.* Response to heparin in patients with ulcerative colitis. *Lancet* 1991; **337**: 238–9.
- 48 Stronkhorst A, Jansen J, ten Berge RJM, *et al.* Functional downregulation and TNF receptor shedding by T-cells following antiCD4 treatment of patients with Crohn's disease [abstract]. *Gastroenterology* 1994; **106**:779.
- 49 Korner H, Goodsall AL, Lemckert FA, *et al.* Unimpaired autoreactive T-cell traffic within the central nervous system during tumor necrosis factor receptor-mediated inhibition of experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* 1995; **92**:11066–70.
- 50 Van deventer SJH. Tumour necrosis factor and Crohn's disease. *Gut* 1997; **40**:443–8.

A national register of HCV infections with a known date of acquisition

A call for study proposals

A national register of hepatitis C virus (HCV) infections with a known date of acquisition is being established, and infections that have been acquired through transfusion of blood or blood components will form the nucleus of the register. It is hoped that the register will provide a national resource for researchers and clinicians alike, and a number of future studies can be envisaged which would benefit from linkage with, and access to, the register.

Mandatory screening of blood donations for antibodies to HCV was introduced by the National Blood Transfusion Service in the United Kingdom in September 1991.¹ As a result, a number of anti-HCV positive donors were identified some of whom had donated blood before the introduction of screening. In early 1995, the UK Health Departments announced that a "look back" at recipients of blood or blood components derived from anti-HCV positive donors would be undertaken.² A high proportion of the recipients of HCV infected blood are expected to be infected with HCV,³ and although most are likely to be asymptomatic in the early stages of infection,⁴ all infected recipients are being referred to clinicians with an interest in hepatic disease for assessment and clinical follow up.

Cases of HCV infection acquired by transfusion and identified by the "look back" are unusual in having a known date of acquisition, an identifiable source, and in having been diagnosed relatively early in the course of infection. Although few in number, the register will be extended to cover other infections of known date, such as documented seroconversions and occupational exposures. Documented seroconversions will include those individuals with virologically confirmed HCV infection for whom a reliable negative result of a test of the same type for HCV infection has been recorded at any point in the preceding four years. The register therefore provides a unique opportunity to monitor incubation period distribution from infection to disease and death, and to provide a group of HCV infections with a known date of acquisition as a resource for other studies.

All clinicians responsible for the care of eligible subjects will be invited to register their patients, and assistance with completion of forms will be available for those clinicians caring for a number of eligible patients. Information on each patient's current clinical status, test results, treatment and management will be gathered using a standard report form, and clinicians responsible for the continuing care of registered patients will be invited to complete and return follow up forms annually on each patient. Such information is urgently needed to help determine the current and future burden of hepatitis C related disease on health care services, and to assess the impact of currently available treatments and those which may become available in the future.

Any researchers interested in applying for access to information held within the national register should contact the register coordinator (details below) for a list of available data. Any study proposals should then be submitted to the register coordinator for consideration by the steering group by 16 October 1998. It is envisaged that the register will provide a national resource that could assist researchers in studies of sexual, vertical and household transmission; clinical trials of new antiviral drugs; further evaluation of existing antivirals and of alternative treatment protocols; determination of the relations between viral load, genotype, treatment and disease progression; and studies of markers prognostic for progression to disease.

NATIONAL HCV REGISTER STEERING GROUP:

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1 CDSC. Hepatitis C and blood transfusion. *Commun Dis Rep CDR Wkly* 1995; **5**:9.

2 Department of Health. Hepatitis C and blood transfusion look back. London: HMSO, 1995 [PL CMO(95)1].

3 Ayob Y, Davidson JI, Baxter A, *et al.* Risk of hepatitis C in patients who received blood from donors subsequently shown to be carriers of hepatitis C virus. *Transfus Med* 1994; **4**:269–72.

4 Seeff LB. Natural History of Hepatitis C. *Hepatology* 1997; **26**(suppl 1):21S–8S.