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Antitumor Necrosis Factor Therapy in Rat Chronic Granulomatous Colitis: Critical Dose-Timing Effects on Outcome[1]

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ABSTRACT

Inhibition of tumor necrosis fact (TNFα) is of potential benefit in the treatment of chronic inflammatory conditions. However, TNFα plays an important role in host defenses against infection, and blocking TNFα production may also have adverse effects. We tested the efficacy and safety of anti-TNFα therapy in experimental colitis induced by trinitrobenzenesulfonic acid. We cultured colonic wall specimens for bacterial growth and measured native TNFα protein synthesis in colonic tissue at days 0, 1, 4, 10 and 18 after induction of colitis. Anti-TNFα therapy (monoclonal g1 immunoglobulin, 15 mg/kg i.p., every third day) was started on either day 4 or day 10 after induction of colitis. On day 18, we measured the release of inflammatory mediators and scored colonic lesions. In acute lesions, several species of the common flora were grown, including *Streptococcus, Staphylococcus, Bacteroides,* clostridia and enterobacteria. In chronic lesions, only enterobacteria, clostridia and lactobacilli were isolated. TNFα production by inflamed colonic tissue was increased in both acute and chronic lesions. Anti-TNFα therapy induced a significant decrease in the release of inflammatory mediators and histopathological remission when treatment started on day 10. However, anti-TNFα therapy increased eicosanoid release and lesion scores when treatment started on day 4. In conclusion, acute colonic lesions showed polymicrobial infection. Anti-TNFα therapy induced remission of chronic intestinal inflammation, but early treatment did not prove effective.

Unrestrained activation of the intestinal immune system appears to be responsible for sustained mucosal inflammation and the characteristic relapsing course of inflammatory bowel diseases (Podolsky, 1991). Interleukin-1β and TNFα are two key cytokines that share a pivotal role in mucosal immunoinflammatory responses. Some studies have shown increased mucosal release of TNFα in patients with inflammatory bowel disease (Breagger *et al.*, 1992; Casellas *et al.*, 1994). Furthermore, expression of TNFα in the mucosa is increased in Crohn's disease (Breese *et al.*, 1994). Hence, Van Dullemen *et al.* (1995) investigated the potential efficacy of an anti-TNFα monoclonal antibody in the treatment of active Crohn's disease and reported a significant benefit in patients with steroid-refractory disease. Recently, controlled clinical trials have also shown a therapeutic effect of antibody neutralization of TNFα in Crohn's disease (Stack *et al.*, 1997; Targan *et al.*, 1997).

TNF-α is produced chiefly by activated macrophages and monocytes (Tracey and Cerami, 1993). This cytokine is involved in the activation of neutrophils (Shalaby *et al.*, 1985), up-regulation of adhesion molecules (Gamble *et al.*, 1985), induction of nitric oxide synthase (Thiemermann *et al.*, 1993), cell-mediated immunity (Bromberg *et al.*, 1992) and granuloma formation (Amiri *et al.*, 1992), among other proinflammatory pathways (Tracey and Cerami, 1993). Most of these effects are mediated by a cascade of inflammatory substances that are induced or released by TNFα, such as platelet activity factor, eicosanoids, nitric oxide, IL-1, IL-6 and IL-8. Therefore, inhibition of TNFα overproduction down-regulates inflammation and is of potential therapeutic benefit. At the same time, however, TNFα plays important roles in host defense against infection. In particular, TNFα is essential for immunity against intracellular pathogens and also exerts antiviral effects (Havell, 1989; Rothe *et al.*, 1993; Wong and Goeddel, 1986). Thus TNFα blockade may have adverse consequences by disarming the organism against pathogens.

Increasing evidence incriminates enteric bacteria in the

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ABBREVIATIONS: TNBS, trinitrobenzenesulfonic acid; TNFα, tumor necrosis factor-α; IL-1, interleukin-1; TXB_2, thromboxane B_2; LTB_4, leukotriene B_4.

pathogenesis of intestinal inflammation. For instance, HLA-B27 transgenic rats spontaneously develop chronic colitis, but rats bred in a germ-free environment fail to develop the disease (Taurog *et al.*, 1994). Likewise, intestinal inflammation in IL-10 knockout (Kühn *et al.*, 1993) mice is dependent on the presence of normal, nonpathogenic intestinal microflora. Strong evidence is also provided by the fact that antibiotics mitigate intestinal inflammation in several animal models, including chronic granulomatous colitis by TNBS (Videla *et al.*, 1994). As shown by our laboratory (García-Lafuente *et al.*, 1997; Videla *et al.*, 1997), bacteria of the common rat flora play a role in the induction of mucosal lesions in TNBS colitis. In this setting, TNFα blockade might compromise mucosal defenses against luminal bacteria.

The aim of the current study was to test the efficacy and safety of anti-TNFα therapy in experimental colitis induced by TNBS. We cultured colonic wall specimens for bacterial growth and measured native TNFα protein synthesis in colonic tissue at various times after induction of colitis. The effect of antibody neutralization of TNFα on colonic inflammation and tissue damage was evaluated.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 220 to 230 g were purchased from CERJ (Le Genest, France). The animals were maintained in a restricted-access room with controlled temperature (23°C) and light-dark cycle (12 h:12 h) and were housed in rack-mounted cages with a maximum of five rats per cage. Standard rodent chow pellets (Biocenter, Barcelona, Spain) and tap water were provided *ad libitum.* The study was approved by the local Research Committee (Comissio de Recerca, Hospital General Vall d'Hebron).

Experimental Design

Colonic wall bacteria in TNBS colitis. Twelve rats were included in this study. Chow pellets were withdrawn for 36 hr, and rats were kept on tap water with 20% sucrose and electrolytes. On day 0, the rats were lightly anesthetized with ether, and colitis ($n = 9$) was induced by intracolonic instillation of 1 ml of a solution containing 60 mg of 2,4,6-TNBS (Sigma Chemical Co., St. Louis, MO) in 20% ethanol (Merck, Darmstadt, Germany) using a rubber cannula (8 cm long, external diameter 2 mm) inserted through the rectum. Control rats ($n = 3$) received 1 ml of saline. Thereafter, chow pellets and tap water were provided *ad libitum*.

Rats were killed by cervical dislocation 4, 10 or 18 days after the intracolonic challenge. Using sterile equipment (surgical instruments, mask, gloves, etc.) a mid-laparotomy was performed, and the distal colon was removed, opened longitudinally and rinsed with sterile saline. In rats with colitis, we selected the ulcerated area for microbiological studies, in control rats a matched area of the distal colon. A 2-cm piece of colon was cut under sterile conditions, washed thoroughly in sterile saline and sonicated twice for 60 sec (Labsonic 2000, Braun, Melsungen, Germany), using each time a new sterile glass container with 3 ml of sterile saline. Specimens were frozen in liquid nitrogen, powdered in a mortar kept at −80°C on dry ice and weighed. Thereafter, samples were homogenized in 9 ml of milk (Difco, Detroit, MI) and stored in aliquots at −80°C for aerobic and anaerobic cultures as described below.

Native TNFα protein synthesis in TNBS colitis. Thirty rats were included in this protocol. Colitis was induced in 20 rats by intracolonic instillation of TNBS, as described above, whereas 10 control rats received intracolonic saline. Rats were killed by cervical dislocation 1, 4, 10 or 18 days after the intracolonic challenge. The distal colon was removed, opened longitudinally and rinsed with normal saline. A 2-cm piece of distal colon was cut, washed thoroughly in saline, homogenized in 2 ml of phosphate-buffered saline (50 mM, pH 7) with 2 mmol/l of phenyl-methyl-sulfonyl fluoride (Sigma) using a Tissue Tearer (model 985-370, Biospec, Racine, WI) and stored at −20°C for later TNFα assay.

Effect of treatment with anti-TNFα antibody on TNBS colitis. Fifty-six rats were included in this protocol. Colitis was induced in all rats by intracolonic administration of TNBS, as described above. Two treatment schedules tested the effect of anti-TNF monoclonal antibody on chronic colitis. In the first schedule, anti-TNF therapy was started on day 4 after TNBS during the acute phase of TNBS colitis (early treatment), and in the second schedule, treatment was started on day 10 after TNBS during the chronic phase of colitis (delayed treatment). As shown by previous studies using this model, histological lesions on day 3 after TNBS instillation consist of extensive coagulative necrosis of the mucosa with epithelial exfoliation, severe submucosal edema and congestion with vascular thrombosis, and acute neutrophil infiltrate (Vilaseca *et al.*, 1990). Chronic changes are observed from 1 to 3 weeks after TNBS (Morris *et al.*, 1989; Vilaseca *et al.*, 1990). Chronic lesions by TNBS are segmental and well circumscribed, consisting of mucosal ulcerations with granulation tissue at the base and mixed transmural inflammation with neutrophils, lymphocytes and macrophages. Small granulomas are observed in the submucosa and serosa. In severe lesions, transmural fibrosis causes stricture of the lumen. Uninvolved areas do not show mucosal inflammation.

Early treatment: The placebo group consisted of 16 rats that received i.p. saline injections on days 4, 7, 10, 13 and 16 after induction of colitis. The anti-TNFα group consisted of 16 rats that received monoclonal anti-mouse TNFα (Chimeric TN3 19.12 g1 from Celltech Research, Slough, UK) in saline at 15 mg/kg as an i.p. injection on days 4, 7, 10, 13 and 16 after induction of colitis. This monoclonal antibody was shown to neutralize native TNFα in Sprague-Dawley rats (Suitters *et al.*, 1994).

Delayed treatment: The placebo group consisted of 12 rats that received i.p. saline injections on days 10, 13 and 16 after induction of colitis. The anti-TNFα group consisted of 12 rats that received monoclonal anti-mouse TNFα (TN3 19.12 g1, Celltech Research) in saline at 15 mg/kg as an i.p. injection on days 10, 13 and 16 after induction of colitis.

Body weight was routinely obtained every second day. On day 18, rats were subjected to intracolonic dialysis under ketamine anesthesia (100 mg/kg i.p.) to measure luminal eicosanoid release. Dialysis bags were prepared with Visking seamless cellulose tubing (8/32, 6.3 mm diameter, 7 cm long; Medicell, London, U.K.) attached *via* an 8-cm rubber cannula to an external syringe. After insertion of the cannula into the distal colon, the dialysis bag was filled with 1 ml of a solution consisting of 0.3% bovine serum albumin in 120 mmol/l NaCl and 30 mmol/l $KHCO_3$. One hour later, the dialysis fluid was recovered and immediately stored at −20°C until eicosanoid assay by specific radioimmunoassay for PGE_2, TXB_2 and LTB_4. Rats were then killed, and the colons were removed and coded for macroscopic and histological assessment of the lesions.

Microbiological Studies

Homogenates from the colonic wall at appropriate dilution volumes were incubated under aerobic or anaerobic conditions. Aerobic media consisted of blood agar, blood agar with nalidixic acid, McConkey agar, CLED agar and Sabouraud agar. Anaerobic media consisted of lacquered blood agar enriched with hemin and vitamin K_1, blood agar with phenyl-ethanol and agar-aztreonam (20 μg/ml). Plates inoculated for obligate anaerobes were incubated in an anaerobic chamber for 48 to 72 hr at 37°C, and plates for aerobes in air at 37°C. After incubation, colonies were identified. Final counts of colonies in cultures of colonic homogenates were referred to a gram of tissue.

Analytical Methods

Eicosanoid concentration in dialysis samples was measured by specific radioimmunoassay for PGE_2, TXB_2 and LTB_4 without prior extraction and HPLC purification (Vilaseca *et al.*, 1990). Tritiated standards were purchased from du Pont de Nemours (Dreiech, Germany), and antisera for PGE_2 and LTB_4 from Advanced Magnetic (Cambridge, MA).

For the TNFα assay, colonic tissue was homogenized with the protease inhibitor phenyl-methyl-sulfonylfluoride (2 mmol/l). Afterwards, samples were centrifuged and the supernatants used for measurement of the concentration of TNFα by a commercial ELISA method for rat TNFα (Biosource, Camarillo, CA). Results are expressed as nanograms of TNFα per gram of wet colonic tissue.

Assessment of Colonic Lesions

The macroscopic lesions were scored by two observers who were unaware of the treatment (JV and FG). A macroscopic score was obtained by summation of scores on severity of colonic adhesions to surrounding tissues, strictures, mucosal ulcerations and wall thickening (table 1). For the histological studies, samples were processed by routine techniques before embedding in paraffin. Sections were obtained from areas showing macroscopic damage, stained with hematoxylin and eosin and coded for blind examination by two pathologist (AS and GG). Both pathologists examined and scored all sections according to the presence of ulcerations, degree of inflammation, depth of the lesions and fibrosis (table 1).

Statistical Methods

Results are presented as mean and S.E.M. Overall statistical difference was determined by one-way analysis of variance, and post-test comparison between treatment and placebo matched group was performed by Student's *t* test. Event rates were compared by Fisher's exact test.

TABLE 1
Morphologic criteria for assessment of colonic damage

	Macroscopic Score	
Adhesions	None	0
	Minimal	1
	Involving several bowel loops	2
Strictures	None	0
	Mild	2
	Severe, proximal dilation	3
Ulcers	None	0
	Linear ulceration < 1 cm length	1
	Two linear ulcers < 1 cm	2
	More sites of ulceration or one large ulcer > 1 cm	3
Wall thickness	Less than 1 mm	0
	1 to 3 mm	1
	More than 3 mm	2
	Maximum possible score	10
	Histological Score	
Ulceration	No ulcer, epithelization	0
	Small ulcers < 3 mm	1
	Large ulcers > 3 mm	2
Inflammation	None	0
	Mild	1
	Moderate	2
	Severe	3
Depth of the lesion	None	0
	Submucosa	1
	Muscularis propria	2
	Serosa	3
Fibrosis	None	0
	Mild	1
	Severe	2
	Maximum possible score	10

Results

Colonic wall bacteria in TNBS colitis. Table 2 shows quantitative bacterial isolates in cultures of colonic wall homogenates from control rats and rats with TNBS colitis. All samples from control colons without macroscopic lesions were positive for *Lactobacillus* species. By contrast, samples from rats with colitis were positive for several bacterial species. As shown, isolates included aerobes (Gram-positive and Gram-negative) and obligate anaerobes that are commonly found among the predominant species of the rat microflora. Interestingly, a number of colonies of *Staphylococcus, Streptococcus* and *Bacteroides* were consistently grown in cultures of colonic lesions at day 4 after TNBS, but *Lactobacillus* were absent. On day 10, however, no growth of *Staphylococcus* species was observed, and *Streptococcus* and *Bacteroides* were found at lower counts than on day 4. Cultures were again positive for *Lactobacillus,* as in control samples. By day 18, no growth of *Staphylococcus, Streptococcus* or *Bacteroides* was detected.

Native TNFα protein synthesis in TNBS colitis. Figure 1 shows TNFα content in colonic homogenates from control rats and rats with colitis induced by TNBS. A significant increase in tissue TNFα concentration was observed on day 1 after induction of colitis, but peak levels were reached on days 4 and 10 after TNBS. In colonic homogenates from rats killed on day 18 after induction of colitis, TNFα concentration returned to base-line levels similar to those found in control rats.

Effect of treatment with anti-TNFα antibody on TNBS colitis. In the first study, five rats from the placebo group and four from the anti-TNFα group died immediately after induction of colitis and before the start of treatment. In addition, one rat from the placebo group and two rats from the anti-TNFα group died during the follow-up, so that by the end of the study there were 10 surviving rats in each group. Both groups of rats manifested a slowing of the rate at which they gained weight after induction of colitis. By day 18, normal growth rates were resumed, and rats treated with the

TABLE 2
Quantitative cultures of colonic wall specimens

Bacterial Species	Cases/Total	Range[a]
	Controls	
Lactobacillus sp.	3/3	1×10^5 to 2×10^5
	Colitis: Day 4	
Escherichia coli	3/3	4×10^6 to 8×10^6
Streptococcus sp.	3/3	2×10^5 to 2×10^6
Staphylococcus sp.[b]	3/3	3×10^5 to 6×10^5
Bacteroides sp.[c]	3/3	6×10^5 to 4×10^6
Clostridium sp.[d]	1/3	4×10^6
	Colitis: Day 10	
Escherichia coli	3/3	1×10^6 to 5×10^6
Streptococcus sp.	3/3	3×10^4 to 7×10^5
Bacteroides sp.[c]	2/3	4×10^4 to 3×10^5
Clostridium sp.[d]	3/3	4×10^4 to 7×10^5
Lactobacillus sp.	3/3	1×10^5 to 6×10^5
	Colitis: Day 18	
Escherichia coli	3/3	4×10^5 to 4×10^6
Klebsiella sp.	2/3	3×10^5 to 4×10^6
Clostridium sp.[d]	2/3	5×10^5 to 6×10^6
Lactobacillus sp.	3/3	4×10^5 to 5×10^6

[a] Colony-forming units per gram of tissue.
[b] Plasma-coagulase negative.
[c] *B. distasonis* in most cases.
[d] *C. barati* in most cases.

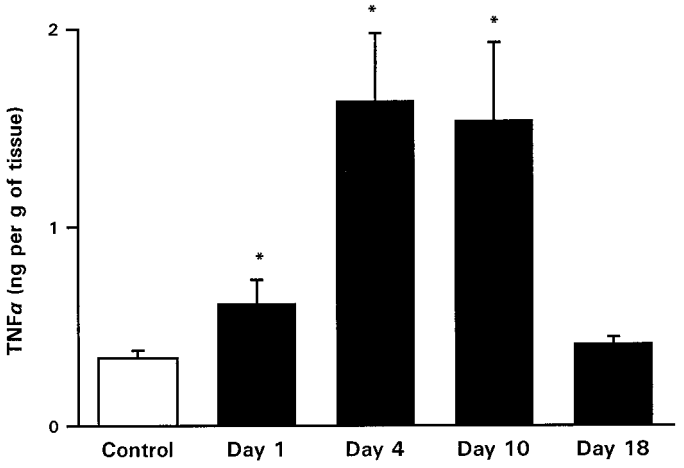

Fig. 1. Content of TNFα protein in colonic homogenates from control rats (open bars) and rats with colitis induced by TNBS (solid bars) (*P < .05 *vs.* controls).

anti-TNFα antibody were heavier (334 ± 11 g) than placebo-treated rats (288 ± 12 g).

In the second study, one rat from each group died before treatments began. Thereafter, one rat from the placebo group died during the follow-up, and at the end of the study there were 10 surviving rats in the placebo group and 11 in the anti-TNFα group. No differences in body weight were found between the two experimental groups (day 18: placebo, 310 ± 20 g; anti-TNFα, 316 ± 14 g).

Figure 2 shows intracolonic release of inflammatory mediators on day 18 after induction of colitis. As shown in the graph at the top of figure 2, the release of PGE_2, TXB_2 and LTB_4 was significantly greater in rats that received early treatment with anti-TNFα antibody than in rats treated with placebo. By contrast, in rats that received delayed treatment with anti-TNFα antibody, the release of inflammatory mediators was significantly lower than in the placebo group (graph at the bottom of figure 2).

The effect of anti-TNFα therapy on morphological lesion scores in chronic TNBS colitis is shown in figure 3. Rats that received anti-TNFα from day 4 after induction of colitis (early treatment) showed significantly higher scores than rats that received placebo. A significant increase in wall thickness was observed in anti-TNFα-treated rats as compared with placebo. Histological scores of ulceration and inflammation were also higher in anti-TNFα-treated rats than in the placebo group.

In contrast, when anti-TNFα therapy was started on day 10 after induction of colitis (delayed treatment), rats showed a significant decrease in morphological lesion scores (fig. 3, bottom). Scores on strictures and wall thickness were significantly lower in anti-TNFα-treated rats than in the placebo group. Likewise, histological scores of ulceration and depth of the lesion were significantly lower in rats that received anti-TNFα therapy.

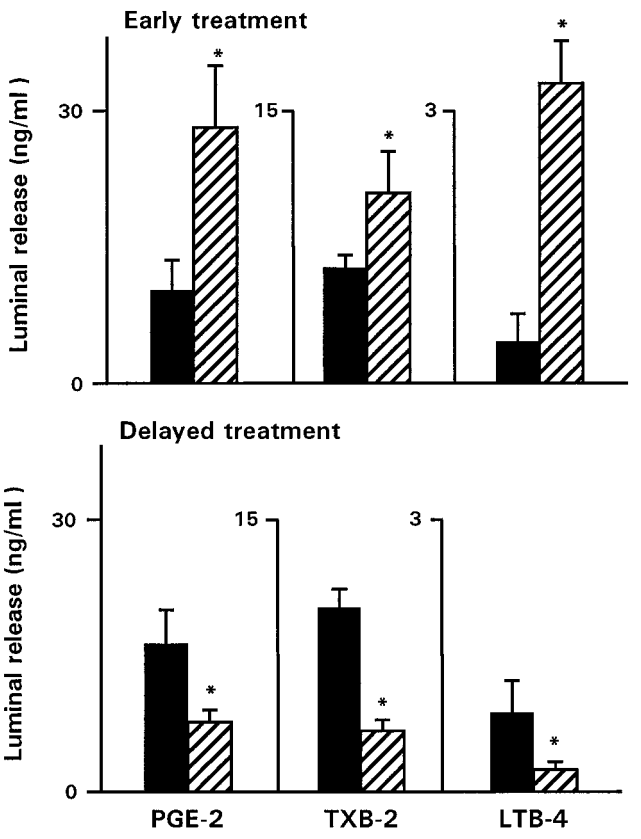

Fig. 2. Intracolonic release of PGE_2, TXB_2 and LTB_4 on day 18 after induction of colitis in rats treated with placebo (solid bars) and in rats treated with anti-TNFα monoclonal antibody (hatched bars). Early treatment was started on day 4 after induction of colitis and continued up to the end of the study. Delayed treatment was started on day 10 after induction of colitis and continued up to the end of the study (*P < .05 between placebo and anti-TNFα rats).

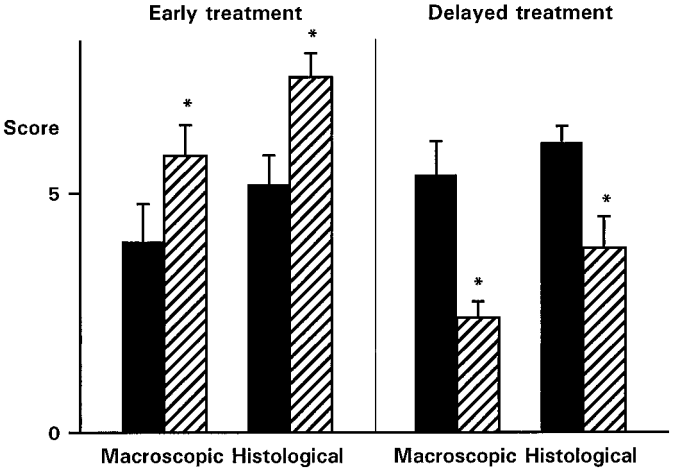

Fig. 3. Morphological scores of colonic lesions on day 18 after induction of colitis in the groups of rats described in figure 2. Macroscopic and histological scores were obtained according to the criteria stated in table 1. Solid bars represent data from placebo-treated rats, and hatched bars represent data from rats treated with anti-TNFα monoclonal antibody (*P < .05 between placebo and anti-TNFα rats).

Discussion

The current study shows that in the early stages of TNBS-induced colitis in the rat, there is superinfection of the colonic lesions with enteric bacteria, including *Streptococcus, Staphylococcus, Bacteroides, Clostridium* and Gram-negative enterobacteria. Conversely, in chronic lesions only Gram-negative enterobacteria, clostridia and lactobacilli are isolated from the colonic wall. Histological evidence for bacterial invasion of inflammatory colonic lesions was previously shown

in experimental colitis (Garcia-Lafuente *et al.*, 1997) and human ulcerative colitis (Ohkusa *et al.*, 1993). The present study suggests that infection of colonic lesions in TNBS-induced colitis is polymicrobial with mixed aerobic and anaerobic organisms. In acute lesions, we found streptococci and other bacterial species, such as *Staphylococcus* and *Bacteroides,* that possess destructive extracellular enzymes. Such histolytic enzymes include phospholipases, nucleases, proteases and collagenases (Bjornson, 1984; Macfarlane *et al.*, 1988). In chronic lesions, however, infection by potentially histolytic species was much less common, and isolates consisted mainly of enterobacteria and lactobacilli. Enterobacteria (Spitz *et al.*, 1994) and lactobacilli (Bernet *et al.*, 1994) are adherent bacteria to the intestinal epithelium and may not directly induce tissue injury. Endotoxin lipopolysaccharide from enterobacteria exerts its biological effects largely by triggering the release of endogenous mediators of inflammation. Thus host responses, rather than intrinsic toxicity, account for most of the damage generated in tissues and organs by endotoxin. Inhibition of the inflammatory response by anticytokine strategies is clearly beneficial in endotoxin-induced shock (Beutler *et al.*, 1985).

Our study shows a different effect of anti-TNFα monoclonal antibody on colonic inflammation, depending on the time of dosing. Anti-TNFα therapy effectively induced biochemical and histopathological remission of colonic inflammatory lesions when initiated beyond the acute phase of the disease. By contrast, anti-TNF therapy proved harmful when started during the early acute flare of the disease. Our data also indicate that native TNFα production in inflamed colonic tissue is enhanced both in the acute phase and the chronic phases and that the outcome of anti-TNFα therapy cannot be predicted by the tissue TNFα levels. Our study therefore suggests that anti-TNFα therapy may be effective and useful in the treatment of chronic intestinal inflammation, whereas acute mucosal lesions may not respond to this therapy.

TNF-α prevents disseminated infection with massive bacteremia in several models (Havell, 1989). Evidence provided by experimental studies indicates that translocation of intestinal bacteria stimulates a systemic TNFα response (Guo *et al.*, 1995) and that anti-TNFα therapy induces dissemination of intestinal bacteria in mice with an injured colonic mucosal barrier (Echtenacher *et al.*, 1990). It was shown that s.c. infection by *Staphylococcus aureus* is aggravated by neutralizing antibodies to TNFα (Vaudaux *et al.*, 1992). A recent study reports that passive immunization against TNFα induces a 4-fold increase in bacterial counts of *Streptococcus pneumoniae* in lung isolates from mice with pneumonia and impairs survival (Van der Poll *et al.*, 1997). In our study, early treatment with anti-TNFα antibody might boost overgrowth of bacteria with histolytic potential within the colonic wall as a consequence of TNFα inhibition and thus expand the area of injury and inflammation. By contrast, delayed treatment would induce truly anti-inflammatory effects, because tissue-destroying bacteria do not appear to participate in chronic stages of colonic inflammation. We acknowledge, however, that further studies are needed to investigate the actual effect of anti-TNFα therapy on bacterial superinfection of inflamed colon.

Other factors may contribute to the contrasting effects of anti-TNFα therapy in acute and chronic colonic lesions. In our study, the placebo group received i.p. injections of normal saline. Likewise, trials on the efficacy and safety of anti-TNFα therapy in patients do not use nonspecific immunoglobulins as a treatment for the placebo group but rather use normal saline (Reinhart *et al.*, 1996), vehicle buffer solution (Van Hensbroek *et al.*, 1996) or human serum albumin (Stack *et al.*, 1997; Targan *et al.*, 1997). Using this approach makes it possible to detect adverse effects due to immunogenic reactions to the exogenous immunoglobulin. Antigen-antibody complexes are known to direct or expand immuno-inflammatory responses either by initiating the complement cascade or *via* interaction with Fc receptors in macrophages, neutrophils and lymphocytes. Formation and deposition of such complexes by neutralization of native TNFα with the monoclonal antibody at sites of inflammation could explain the inflammatory burst observed in our experiments when treatment was started in the acute phase of colitis. However, the antibody isotype is critical in determining immune complex-mediated inflammatory responses. Hence, immune complexes that contain murine immunoglobulines of the g1 isotype do not bind to Fc receptors or complement fraction 1q, and they are inactive. In fact, previous studies with the same antibody have shown that *in vivo* neutralization of native TNFα in Sprague-Dawley rats did not induce immune complex-associated responses, as compared with the g2a isotype (Suitters *et al.*, 1994). It is therefore unlikely that in our experiments, anti-TNFα therapy exacerbated colitis because of immune complex-mediated reactions. However, immunogenic reactions to the antibody cannot be totally excluded. The fact that rats subjected to the early-treatment schedule received five doses of the antibody, whereas rats subjected to delayed treatment received only three doses, may have influenced the outcome. Nevertheless, such adverse effects were not significant in human studies with repeated doses of the antibody (Reinhart *et al.*, 1996) or higher doses (20 mg/kg; Targan *et al.*, 1997).

It is interesting to note that anti-TNFα therapy was associated with increased body weight gain in rats subjected to the early-treatment schedule as compared with control rats, despite a higher release of inflammatory markers and more severe lesion scores in anti-TNFα-treated rats than in controls. TNFα has been implicated as humoral mediator of cachexia associated with infection and inflammation (Tracey and Cerami, 1993), and the effect on body weight gain may be due to blockade of the native cytokine. In our experiment using the delayed-treatment schedule, this effect was not observed. This group of rats received three doses of the anti-TNFα antibody, whereas rats subjected to early treatment received five doses of the antibody.

Anticytokine strategies have previously been shown to be useful in experimental colitis. Beneficial effects of IL-1 receptor antagonists on intestinal inflammation were observed in rabbit immune complex colitis (Cominelli *et al.*, 1990). The current study suggests that neutralization of TNFα with monoclonal antibodies is an effective tool in the treatment of chronic intestinal inflammation. As we learned from our experimental model, caution must be exercised in the treatment during the acute destructive phase, because the balance between defense against bacterial aggression and organ inflammation may be altered inappropriately and precipitate a net unfavorable outcome.

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