Tumour necrosis factor α changes porcine intestinal ion transport through a paracrine mechanism involving prostaglandins

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Abstract

Prostaglandins stimulate electrogenic anion secretion and inhibit sodium chloride absorption in cryptosporidium induced pig diarrhoea. Because tumour necrosis factor α (TNF α) is an early mediator of inflammation and stimulates prostaglandin secretion, we investigated its effect on intestinal ion transport. Cryptosporidium infected pig ileum showed higher macrophage infiltration and tissue TNF α -like activity than uninfected tissues ($p < 0.05$, $n = 4$ and $p < 0.05$, $n = 12$, respectively). TNF α treatment of control porcine ileal mucosa increased the short circuit current (Isc), a measurement of net anion secretion in this model ($p < 0.001$, $n = 23$). This effect was blocked by 10^{-6} M indomethacin and Cl^- replacement. Neither acute treatment nor preincubation of colonic intestinal epithelial cell monolayers (T84) with TNF α stimulated the Isc. However, co-mounting of TNF α preincubated pig jejunal fibroblasts (P2JF) monolayers back to back with untreated T84 monolayers dose-dependently induced an indomethacin sensitive increase in Isc compared with values in untreated co-mounted monolayers ($p < 0.001$, $n = 11$). These data suggest that in infectious diarrhoea, TNF α may induce Cl^- secretion through a paracrine mechanism involving prostaglandin release from subepithelial cells, for example fibroblasts.

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Recent evidence indicates an intimate interaction between the immune, enteric-neural, and endocrine systems and mesenchymal cells in the regulation of intestinal water and electrolyte transport.^{1,2} Several mediators of immune and inflammatory reactions, such as prostaglandins and reactive oxygen metabolites, stimulate Cl^- secretion and inhibit NaCl absorption in a colonic epithelial cell line, pig ileum, and rat colon.¹⁻⁶ Increased prostaglandin concentrations in the ileum of cryptosporidium infected pigs have recently been implicated in the pathophysiology of diarrhoea induced in this model through the inhibition of NaCl absorption and stimulation of Cl^- secretion.⁷

The cytokine, tumour necrosis factor α (TNF α), a 17 kDa protein secreted mainly by macrophages,⁸ modulates various functions of cells involved in inflammatory reactions, host

defense, and tissue repair. It is believed to be a key mediator of inflammatory reactions and may be a very proximal step in the immune reaction cascade. It is a chemoattractant of neutrophils and macrophages,^{9,10} and induces the secretion of prostaglandins and reactive oxygen metabolites from several cell lines such as human skin fibroblasts and endothelial and mesangial cells.¹¹⁻¹⁷ Several reports indicate an increase in TNF α protein and mRNA in patients with inflammatory bowel disease,¹⁸⁻²¹ although similar changes were undetectable in other studies.²²⁻²⁴ One of the reasons for this inconsistency may be the degradation of TNF α by proteases in inflamed tissue.²⁵ The effect of TNF α on intestinal ion transport and its role in the pathogenesis of infectious diarrhoea, however, have not been reported.

Assuming that TNF α stimulates prostaglandin secretion from intestinal cells as it does from cells of other tissues, we suggest that increased TNF α values in infections and inflammation modulate intestinal electrolyte transport. Moreover, as the lamina propria and submucosa are the main source of prostaglandin,²⁶⁻²⁸ we postulated that the effect of TNF α on electrolyte fluxes across the intestinal epithelium is mediated through the release of other inflammatory mediators, such as prostaglandin, from subepithelial mesenchymal cells which subsequently modulate epithelial cell function in a paracrine fashion.

Methods

REAGENTS AND ANIMALS

Recombinant human TNF α (rTNF α) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, In 46250) and primary monoclonal anti-porcine macrophage antibody from American Type Culture Collection (Rockville, MD). Zymed streptavidin-biotin system for immunohistological staining of macrophages was obtained from Zymed Laboratories Inc (San Francisco, CA 94080) and human TNF α ELISA kit from Genzyme Corporation (Cambridge, MA 02139). All other chemicals were obtained from Sigma Chemical Co (St Louis, MO 63178).

Study piglets were obtained from the CGIBD Barrier Intact Piglet Facility at NCSU, College of Agriculture. Animals were prevented access to the sow after birth to prevent transmission of passive immunisation and were fed 12.5 ml/kg/h artificial formula with an automatic feeding device (Autosow).²⁹

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Some animals were infected with 10^8 *Cryptosporidium parvum* oocysts on day 3 of life as previously described.³⁰ Rectal swabs were taken daily for determination of cryptosporidia oocysts and rotavirus.³⁰ All infected animal stools were positive for cryptosporidia and showed histological indication of infection.³⁰ All animal stools were negative for rotavirus and control animal stools were negative for cryptosporidia. Both infected and control animals were killed three to five days after inoculation (peak infection) by intracardiac injection of 2–4 g of sodium pentobarbital. Preanaesthesia with 50–70 mg intramuscular ketamine was given.

TNF α EXTRACTION

After stripping the muscle layer, 4 cm of ileum were homogenised, in phosphate buffered saline (PBS) with 1% bovine serum albumin and 1 μ g/ml of each of antipain, aprotinin, leupeptin and pepstatin A, and 1 mM PMSF as previously described for interleukin 1 extraction.³¹ An ELISA using anti-rTNF α antibody was used to determine porcine TNF α values. rTNF α has 80% homology with porcine TNF α , and anti-rTNF α has been reported to cross react with porcine TNF α , blocking 97% of its activity.^{32 33}

To validate the extraction procedure, we determined the recovery of rTNF α (150, 300, or 600 pg/ml) added to homogenised ileal tissues from control animals. The mean (SEM) recovery was 73 (10)%, compared with 36 (18)% when tissues were homogenised in PBS (n=12). The observed TNF α value was highly correlated with the expected concentrations, indicating the absence of any significant interference (R=0.84).

MACROPHAGE COUNTING

Macrophages were immunohistologically stained utilising the mouse IgG1; 74-22-15 as the primary anti-porcine macrophage antibody.³⁴ The number of macrophages and the surface area of five consecutive lamina propriae from each histological slide were measured by the same observer, who was blinded according to the experimental protocol. Measurements were done with a video imaging morphometric system (Image-Pro II, Media Cybernetics, Silver Spring, MD). Images were captured from a Nikon Microphot-FXA with a Javelin 3452RGB television camera.

IN VITRO TRANSPORT STUDIES

The distal 25 cm of the ileum were removed, submerged in Ringer's solution, opened along the antimesenteric border for stripping of the muscle layer, and then mounted on Ussing chambers exposing 1.13 cm². For tissues used to test the effect of indomethacin on the TNF α effect, stripping was carried out in oxygenated buffer with 1 μ M indomethacin. As an energy source, 10 mM glutamine was added to the serosal side and equimolar mannitol on the

mucosal side of mounted tissues. The buffer contained (mM) 140 Na⁺, 5.2 K⁺, 1.2 Ca⁺⁺, 1.2 Mg⁺⁺, 119.8 Cl⁻, 25 HCO₃⁻, 0.4 H₂PO₄⁻, and 2.4 HPO₄⁻⁻, pH 7.4 when gassed with 95% O₂/5% CO₂. Bathing solutions were circulated through a water jacket and were maintained at 37°C. The spontaneous potential difference (PD) was short circuited by an automatic voltage clamp (World Precision Instruments, New Haven, CN) that corrected for fluid resistance. Tissues were continuously short circuited (except when the spontaneous PD was read every 30 minutes) using Ag-AgCl electrodes connected to bathing solutions via agar bridges. Short circuit current (Isc), which is the current automatically applied to short circuit the PD to zero, was recorded every 10 minutes. Conductance (G) was calculated based on Ohm's law using Isc and spontaneous PD or, if PD was $\leq \pm 1$ mV, a clamped Isc of ± 100 μ A and the corresponding PD. Tissues were allowed to equilibrate for 20 or 90 minutes before early and late experiments, respectively.

rTNF α was added to the serosal side of ileal segments in the Ussing chamber and compared with matched control tissue from adjacent ileal segments from the same animal. A 10^{-6} M serosal prostaglandin E₂ (PGE₂) treated tissue was included in some studies to test the ability of the tissue to respond to secretory stimuli. In addition, at the end of each study, the effect of 10 mM mucosal glucose on the Isc was measured as an indication of tissue viability. The maximum increases in Isc from the pretreatment level (Isc-max) in response to TNF α and PGE₂ during a 60 minute follow up period were compared with the corresponding changes in time matched control tissues from the same animal.

CELL LINES AND CULTURE TECHNIQUES

T-84 cells, an established colonic carcinoma cell model of active intestinal Cl⁻ secretion,³⁵ and pig jejunal fibroblasts, P2JF,³⁶ were plated in 100 mm² Corning tissue culture plates at a seeding density of about 10^6 cells per plate and incubated at 37°C in 5% CO₂-95% air with 98% humidity in a water jacketed incubator. The growth media for T-84 cells was Dulbecco's minimum essential media (DMEM/F12) with 5% fetal calf serum (FCS). For P2JF fibroblast cells, DMEM/F12 with 10% FCS was used. Media were replaced every three to four days. Upon reaching confluence, the cell monolayers were subcultured (1:3) using 0.1% trypsin-1.4 mM EDTA solution, every seven to 14 days. For electrical studies, cells were trypsinised and then plated on 25 mm polycarbonate filters, with 5 μ m pores (Nucleopore filters, VWR Scientific) which had been coated with rat tail collagen and glued to plastic rings.^{35 37} One ml of medium, containing approximately 10^6 cells, was placed in each ring structure with a surface area of 2.98 cm². The rings were supported on 3 mm glass beads in culture dishes containing the appropriate culture medium, which was

TABLE 1 Increased tumour necrosis factor α (TNF α) immune activity and macrophage infiltration in the ileum in cryptosporidium induced diarrhoea

	Macrophages/mm ² of lamina propria	TNF α (pg/mg protein)
Control (mean (SEM))	50 $\pm$ 26	297 (34)
Infected (mean (SEM))	478 (153)*	420 (48)*

Frozen sections were obtained from the terminal ileum of control and infected piglets and immunohistologically stained for macrophages using the streptavidin-biotin method (n=4). For determination of tissue TNF α like activity, 4 cm of stripped ileum were homogenised in PBS with 1% BSA and a mixture of antiproteases (see text). TNF α -like activity was measured by an ELISA using anti-rTNF α antibody (n=12). *Data are different from control, p<0.05.

replaced inside and outside the rings every three to four days. Cells were incubated on the rings for seven to 14 days before experimentation. Ring structures with the cultured cell monolayers were mounted in modified Ussing chambers³⁵ and the Isc and PD were followed every 10 minutes as described above.

To determine the importance of fibroblasts to the effect of TNF α on the epithelial cell line, monolayers of fibroblasts and epithelial cells were mounted back to back on Ussing chambers so that cell monolayers were spatially separated by 1 mm but were in contact through the incubation buffer present between the two ring structures.³⁶ Cell monolayers were incubated for 24 hours with TNF α (0.05 to 50 ng/ml). After a 40 minute equilibration period, the Isc and PD were recorded for 20 minutes.

STATISTICS

Data are presented as the maximum change in Isc from pretreatment level in response to treatment (Isc-max). Δ Isc-max is the difference between Isc-max of treated and time matched control tissues from the same animal. Comparisons of paired data were conducted with a paired *t* test. A p value of <0.05 was considered significant. Data are presented as mean (SEM).

Results

TNF α INVOLVEMENT IN CRYPTOSPORIDIOSIS DIARRHOEA

As a preliminary indication of the role of TNF α in the development of infectious diarrhoea we determined the number of macrophages, the main cellular source of TNF α , in the lamina propria and TNF α like activity in the ileum of cryptosporidium infected pigs versus age and litter matched control animals. As shown in Table I, there

was a significant increase in the concentration of macrophages and TNF α like activity in the ileum of infected animals over control levels (p<0.05).

TISSUE VIABILITY

As summarised in Table II, there was no change in tissue conductance during the three hour period of the study, indicating stability of the tissue in vitro. Tissues that showed conductance outside the 95% confidence interval (95% CI) around the mean conductance at baseline were excluded from further analysis. Serosal addition of 10⁻⁶ M PGE₂ to ileal tissues from nine animals one hour after mounting resulted in a Isc-max of 55 (11) μ A/cm². This was not significantly different from the response when PGE₂ was added 20 minutes after mounting (73 (7) μ A/cm²), indicating that the tissue's ability to respond to secretory stimuli was maintained throughout the study. There was, however, a reduction in tissue response to the serosal application of 10⁻⁶ M PGE₂ after three hours (Isc-max=21 (10) μ A/cm², p<0.01). All tissues consistently responded to 10 mM mucosal glucose added

Figure 1: Effect of 25 ng/ml tumour necrosis factor α (TNF α) added 20 minutes after mounting on the increase in short circuit current (Δ Isc) of normal pig ileum. TNF α or PGE₂ (10⁻⁶) were added to the serosal side of stripped ileal tissues and compared with control tissues from the same animal. Isc-max is the maximal change of Isc from pretreatment values in response to treatment. Δ Isc-max is the difference between Isc-max of treated and time matched control tissues from the same animal. Data are mean (SEM). Isc changes in response to TNF α and prostaglandin PGE₂ in a representative animal are presented in Figure 1B. *Statistically different from control, p<0.05.

TABLE II Effect of 25 ng/ml tumour necrosis factor α (TNF α) on the conductance of normal pig ileum

Group	Time (min) after TNF α treatment (msiemens/cm ²)			
	0	60	120	180
Control (mean (SEM))	21.3 (1.6)	19.0 (2.0)	20.0 (2.0)	24.8 (5.5)
TNF α (mean (SEM))	21.4 (1.7)	20.1 (1.7)	19.0 (1.8)	20.5 (4.1)

TNF α was added to the serosal side of normal piglet ileal tissues 20 minutes after mounting on Ussing chambers. Data are TNF α treated and time matched control tissues from the same animal (n=7).

Figure 2: ΔI_{sc-max} dose response of tumour necrosis factor α (TNF α) in normal pig ileum: The indicated doses of TNF α were added to the serosal side of stripped ileal tissue segments from the same animal 20 minutes after mounting on Ussing chambers. I_{sc-max} is the maximal change of I_{sc} from pretreatment values in response to treatment. ΔI_{sc-max} is the difference between I_{sc-max} of treated and time matched control tissues from the same animal. Data are mean (SEM).

at the end of each experiment with an increase in I_{sc} of more than 20 $\mu A/cm^2$.

EFFECT OF TNF α ON ION TRANSPORT

When the piglet ileum is stimulated with PGE $_2$, there is stimulation of active electrogenic Cl $^-$ and HCO $_3^-$ secretion, reflected by an increase in I_{sc} , as well as inhibition of electroneutral NaCl absorption.^{4,7} Figure 1 shows the effect of 25 ng/ml serosal rTNF α (2×10^{-7} M) on the I_{sc} of normal piglet ileal tissue when added 20 minutes after mounting. TNF α treatment resulted in a significant increase in I_{sc-max} above time matched control levels (42 (6) v 12 (3) $\mu A/cm^2$). The TNF α effect was transient and was observed 30 (2.9) minutes after treatment, compared with a PGE $_2$ effect of 58 (10) $\mu A/cm^2$ consistently observed 10 minutes after treatment (Fig 1). In a graded dose response study, adding 0.05 to 50 ng/ml TNF α to the serosal bathing solution showed a threshold effect between 0.5 and 5 ng/ml (Fig 2).

As shown in Figure 3, the TNF α effect was blocked by indomethacin (10^{-6} M) added to both sides of the tissue before treatment with TNF α ($p < 0.01$, $n = 5$) and by Cl $^-$ replacement from the bathing buffer ($p < 0.01$, $n = 3$), indicating that the TNF α stimulation of I_{sc} was Cl $^-$ dependent and related to the

Figure 3: Effect of indomethacin (10^{-6} M) and Cl $^-$ replacement on 25 ng/ml tumour necrosis factor α (TNF α) mediated increase in short circuit current (I_{sc}) in normal pig ileum: TNF α was added to the serosal side of stripped ileal tissue segments from the same animal incubated in normal Ringer's solution, normal Ringer's with 10^{-6} M indomethacin or Cl $^-$ free buffer. I_{sc-max} is the maximal change of I_{sc} from pretreatment values in response to treatment. ΔI_{sc-max} is the difference between I_{sc-max} of treated and time matched control tissues from the same animal incubated in the same buffer. Data are mean (SEM).

cyclo-oxygenase metabolites of arachidonic acid.

SITE OF TNF α EFFECT

We were interested in determining whether TNF α exerts any direct effect on intestinal epithelium or acts mainly through its effect on other mesenchymal tissues. Direct addition of TNF α to mounted T-84 cell monolayers or fibroblast monolayers comounted with T-84 monolayers had no effect on I_{sc} for up to three hours follow up (data not shown). Parallel studies indicated that TNF α started to induce P2JF production of PGE $_2$ after 24 hours' incubation (Dr Berschneider; unpublished data). We next compared the I_{sc} across T-84 cell monolayers mounted on Ussing chambers alone or back to back with P2JF monolayers (T84/P2JF) after 24 hours' preincubation with 25 ng/ml TNF α of either the T-84 or the P2JF monolayers. Basal I_{sc} was followed for 20 minutes, beginning 40 minutes after mounting, when it reached a steady state. As shown in Figure 4, TNF α preincubation of the T84 monolayers had no effect on the basal I_{sc} across the T84 monolayers, whether mounted alone or with P2JF monolayers. On the other hand, preincubation of the P2JF monolayers induced an increase in basal I_{sc} across the T84/P2JF monolayers compared with that across untreated T84/P2JF monolayers (9.0 (1.3) v 3.9 (0.4) $\mu A/cm^2$, $p < 0.001$). As was the case with pig ileal tissue, the TNF α induced increase in I_{sc} was blocked with 10^{-6} M indomethacin indicating the involvement of

Figure 4: Effect of incubation of T-84-cell or fibroblast monolayers with TNFα for 24 hours before mounting on Ussing chambers on short circuit current (Isc) across T-84/PJ2F. For 24 hours before mounting, either T-84 cell or fibroblast monolayers were incubated with TNFα (25 ng/ml). After 40 minutes' equilibration, Isc was followed for 20 minutes. Isc was averaged over the 20 minute follow up period. Data are mean (SEM). *Statistically different from control, T84 (no TNF) + P2JF (no TNF), p<0.05.

cyclo-oxygenase metabolites of arachidonic acid in mediating TNFα effect (Fig 4).

DOSE RESPONSE OF TNFα ON ISC OF T-84/FIBROBLASTS

Monolayers of fibroblasts were incubated with 0.01 to 50 ng/ml TNFα for 24 hours before mounting back to back with untreated T-84 cell monolayers. As shown in Figure 5, a threshold between 0.5 and 1 ng/ml was observed with a maximum increase of basal Isc at 25 ng/ml TNFα.

Discussion

To our knowledge this is the first study to show

Figure 5: Dose effect of 24 hours' incubation of fibroblast monolayers with tumour necrosis factor α (TNFα) on short circuit current (Isc) across T-84/PJ2F. Each group consisted of T-84 cell monolayers mounted on Ussing chambers back to back with fibroblast (PJ2F) monolayers (T-84/PJ2F). For 24 hours before mounting, fibroblast monolayers were incubated with the indicated TNFα doses. After 40 minutes' equilibration, Isc was followed for 20 minutes. Isc was averaged over the 20 minute follow up period. Adjusted Isc is the difference between Isc of treatment and control T-84/PJ2F monolayers. Data are presented as mean (SEM).

the effect of TNFα on intestinal epithelial cell ion transport. Our data show that serosal application of rTNFα to normal piglet ileal tissues mounted on Ussing chambers stimulated an increase in Isc that was significantly higher than that observed for control ileal tissues from the same animals, indicating a TNFα effect on electrogenic ion transport. These data, in addition to the increased TNFα-like activity and macrophage infiltration in cryptosporidium induced diarrhoea, suggest a role for TNFα in the diarrhoea developed in this model.

The pathophysiological role of TNFα in intestinal tissue inflammation is still controversial. Some reports indicate an increase in TNFα protein and mRNA in patients with inflammatory bowel disease,¹⁸⁻²¹ although similar changes were undetectable in other studies.²²⁻²⁴ It has been suggested that the inability to detect any TNFα increase in the latter reports may be due to the following: (a) therapy, such as corticosteroids, that may inhibit TNFα level; (b) the method of TNFα measurement; Olson *et al*¹⁹ used immuno-histochemical staining rather than measurement of TNFα in homogenised tissue; (c) the possibility that younger subjects express more TNFα; and (d) TNFα degradation by proteases in inflamed tissue.²⁵ Our data indicate that the addition of antiproteases to homogenisation buffer is important for the detection of tissue TNFα activity.

Several reports indicate a stimulatory effect of TNFα on the secretion of prostaglandins,^{11 15-17} although the TNFα effect on intestinal production of prostaglandins has not been previously reported. Our data indicate the involvement of prostaglandins in the TNFα mediated effect on Isc. Firstly, most of the TNFα mediated changes in ion transport, both in ileal tissues and T-84 cell/fibroblast model, were blocked by 10⁻⁶ M indomethacin, a blocker of prostaglandin synthesis through inhibition of cyclo-oxygenase enzyme. Although indomethacin has been reported to have unspecific effects other than cyclo-oxygenase inhibition, such as inhibiting K⁺ stimulated ATPase and Ca⁺⁺ uptake, higher concentrations have been required to elicit these effects than those needed for the inhibition of prostaglandin production.^{39 40} Secondly, incubation of the porcine intestinal fibroblasts, P2JF, with TNFα significantly increased the PGE₂ level in the culture media (Dr Berschneider; unpublished data), further indicating prostaglandin involvement in the TNFα effect.

The increase of Isc in response to TNFα is most probably mediated by electrogenic Cl⁻ secretion. Firstly, the PGE₂ induced increase of Isc in piglet ileum has previously been shown to be mediated by electrogenic Cl⁻ secretion.^{4 7} Secondly, Cl⁻ replacement from the bathing buffer blocked the TNFα stimulation of Isc. Thirdly, the observed increase of Isc in T84 cells generally reflects electrogenic Cl⁻ secretion.³⁵

Our data indicate that the effect of TNFα on epithelial cells is indirect. TNFα preincubation

Figure 6: Proposed mechanism of $\text{TNF}\alpha$ modulation of ion transport across the intestinal epithelial cells. We postulate a chain of events where: (1) $\text{TNF}\alpha$ and probably other cytokines are released early in intestinal infectious and inflammatory disorders. (2) Cytokines act on subepithelial intestinal cells to release other inflammatory mediators such as prostaglandins. (3) Prostaglandins act on the intestinal epithelial cells changing ion transport and inducing diarrhoea. Modified from Bern et al. (*J Clin Invest* 1989; 83: 1810–20.)

of the epithelial cell line T-84 had no effect on Isc whether mounted alone or co-mounted with untreated control fibroblast monolayers. It was only when T-84 cell monolayers were co-mounted with fibroblast monolayers which had been preincubated with $\text{TNF}\alpha$ for 24 hours, that an increase in Isc was observed. Because co-mounted monolayers in this system do not have any direct contact³⁷ the increase in Isc across epithelial cells co-mounted with fibroblasts is mediated by products released from fibroblasts in response to preincubation with $\text{TNF}\alpha$. Major candidates for mediating this effect are prostaglandins, although other mediators may also be involved. This indicates an important intercellular communication in mediating the $\text{TNF}\alpha$ effect on intestinal epithelial cells. Intercellular communication has recently been reported in cells from the same line – that is, glomerular mesangial cells, where cells activated with $\text{TNF}\alpha$ may release stimulant(s) that affect adjacent cells^{41 42} and a paracrine modulation of the intestinal and renal epithelial function by fibroblasts and endothelial cells, respectively, has been suggested.^{35 37} In addition, the data by Lawson et al, Smith et al, and Craven et al^{26–28} indicating that most of the prostaglandin production is in the submucosa, whereas the prostaglandin effect is mainly on the epithelium, supports the existence of such a paracrine mode of action in the intestine.

There is growing evidence for the importance of interactions between different inflammatory mediators in the development of maximum immune mediated reactions. This

'priming' effect has been reported between $\text{TNF}\alpha$ and other cytokines such as interleukin 1 (IL-1).^{11 45 46} Recent preliminary data by Berschneider and Goralska indicate the presence of a synergistic interaction between $\text{TNF}\alpha$ and IL-1 in stimulating increased Isc across T-84 cells.⁴⁷ Synergistic interaction between $\text{TNF}\alpha$ and IL-1 in stimulating the fibroblast secretion of PGE_2 has been reported¹¹ and P2JF cells primed with IL-1 show a faster induction of PGE_2 synthesis in response to $\text{TNF}\alpha$ than unprimed cells (Dr Berschneider; unpublished data). Priming or synergistic interaction between $\text{TNF}\alpha$ and the immune cells, other cytokines, or the sub-mucosal enteric nerve plexuses may be the reason for the faster response of whole tissues than that of the cell culture model to $\text{TNF}\alpha$ in our study.

In conclusion, we suggest a role for $\text{TNF}\alpha$ in the pathophysiology of infectious diarrhoea induced in a model of cryptosporidiosis infection in the pig. We postulate a chain of events where: (1) $\text{TNF}\alpha$ and probably other cytokines are released early in intestinal infectious and inflammatory disorders; (2) cytokines act on subepithelial intestinal cells to release other inflammatory mediators such as prostaglandins; and (3) prostaglandins act on the intestinal epithelial cells altering ion transport and inducing diarrhoea (Fig 6).

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