SMALL INTESTINE

Neutrophil migration into indomethacin induced rat small intestinal injury is CD11a/CD18 and CD11b/CD18 co-dependent

A W Stadnyk, C Dollard, T B Issekutz, A C Issekutz

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See end of article for authors' affiliations

Correspondence to: A W Stadnyk, Infection and Immunology Research Laboratories, IWK Health Centre, 5850 University Avenue, Halifax, Nova Scotia, Canada B3J 3G9; Andrew.Stadnyk@Dal.Ca

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Background: Neutrophils may exacerbate intestinal inflammatory diseases through secretion of proteolytic enzymes and reactive oxygen and nitrogen intermediates.
Aims: To define the mechanisms involved in neutrophil infiltration into the non-steroidal anti-inflammatory disease inflamed intestine to develop strategies to regulate this process.
Methods: The small intestinal epithelium of (15 mg/kg) indomethacin treated rats was examined for cytokine mRNA. The kinetics of neutrophil accumulation into the gastrointestinal tract (including lumen contents) of inflamed rats was determined using radiolabelled (^{111}In) neutrophils injected intravenously followed by a three hour migration period. To determine which adhesion molecules were critical for migration, rats were also injected with function blocking monoclonal antibodies to the β_2 (CD11/CD18) integrins.
Results: Interleukin 1β, interleukin 1 receptor II, tumour necrosis factor α, and monocyte inflammatory peptide 2 but not monocyte chemoattractant protein 1 mRNA were detected in the epithelium within hours of indomethacin injection. Neutrophils were detectable in the small intestine and intestinal lumen by six hours and continued to accumulate until 48 hours post indomethacin injection. Neutrophil accumulation in the intestine was essentially blocked by anti-CD18, and partially blocked by either anti-CD11a or CD11b antibody treatment. Migration into the intestinal lumen was reduced by anti-CD11b.
Conclusions: The small intestinal epithelium acts as one source of cytokines with properties important in the recruitment of neutrophils. In turn, neutrophil migration into the indomethacin inflamed small intestine is mediated by CD11a/CD18 and CD11b/CD18.

Various rodent models of intestinal disease exist which collectively are intended to provide insights into the mechanisms underlying human intestinal inflammation, and in many cases human inflammatory bowel disease.[1] To this end we have been studying the rat host response to the small intestinal parasite, *Trichinella spiralis*. Our results implicate the small intestinal epithelial cells (IEC) as a significant source of early cytokines, including interleukin 1β (IL-1β), indirectly important in the recruitment of leucocytes.[2] More recently, we discovered that IEC make the potent neutrophil chemoattractant monocyte inflammatory peptide (MIP)-2, and that neutrophils infiltrate the gut during infection.[3] For the purpose of determining whether there is a single common pathway to inflammation in the small intestine we have begun to compare the host response with IEC cytokines and pattern of neutrophil migration in a second rat model exhibiting mucosal injury without infection—that is, small intestinal injury following indomethacin injection.

A single injection of indomethacin induces a transient gastric injury followed by small intestinal inflammation, including sloughing of the epithelium and ulcerations.[4–7] While much of the focus of research into this response has been on gastric events, we were intrigued by similarities between the small intestinal mucosal inflammation and the helminth infection. Furthermore, while the pattern of neutrophil infiltration and adhesion molecules important for migration into the stomach has been studied,[4 5 8 9] epithelial cytokines and neutrophil infiltration into the small intestine are less well characterised.

Neutrophil infiltration into sites of inflammation is the outcome of a combination of chemoattractant signals and increased expression of adhesion molecules on the local vascular endothelium. Neutrophil transendothelial migration is staged into a series of events: initial rolling (primarily selectin mediated), activation (primarily chemoattractant dependent), firm adhesion (integrin mediated), and transmigration (utilising integrins and immunoglobulin superfamily molecules, for example, PECAM).[10–12] Migration of neutrophils into the intestine has been shown to be largely CD18 dependent (β_2 integrin) in some models of inflammation as treating animals with function blocking monoclonal antibodies (mAbs) blunts the increase in myeloperoxidase (MPO) levels otherwise seen in untreated inflamed animals.[13] The CD18 chain can pair with one of four distinct α chains on leucocytes, α_L (CD11a, yielding lymphocyte function associated molecule 1 (LFA-1), α_M (CD11b, yielding Mac-1), α_x (CD11c, yielding p150/95), or α_D (CD11d)[12] and thus a number of integrins with differing ligand specificities are available for mediating transendothelial migration. In non-intestinal models of inflammatory diseases the role of the individual CD11/CD18 integrins in leucocyte migration varies with the inducing agent, the tissue site, and tissue compartment but utilisation of the different CD11 chains is not well characterised in intestinal inflammation. Understanding which CD11 chain(s) are used is important if we expect to develop therapies intended to block neutrophil

Abbreviations: IEC, intestinal epithelial cells; IL-1β, interleukin 1β; IL-1RII, interleukin 1 receptor II; MIP-2, monocyte inflammatory peptide 2; mAb, monoclonal antibody; MPO, myeloperoxidase; PBS, phosphate buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; TNF-α, tumour necrosis factor α; rbc, red blood cells; LFA-1, lymphocyte function associated molecule 1.

migration into inflammatory sites. Therefore, our objective was to determine, in indomethacin injury, first whether IEC make cytokines relevant to neutrophil recruitment and if so, the pattern of neutrophil migration into the inflamed small intestine, including the extent to which CD11/CD18 molecules are involved.

MATERIALS AND METHODS

Animals and injury

Non-fasted male Lewis rats (Harlan Sprague-Dawley, Indianapolis, Indiana, USA) were administered a single subcutaneous injection of indomethacin (Sigma Chemical Co., St Louis, Missouri, USA) dissolved in freshly prepared and sterile filtered 5% sodium bicarbonate (Baker Inc., Phillipsburg, New Jersey, USA). Rats were injected in the early afternoon. All animals used for collection of tissues showed macroscopic ulcers but there was no mortality. All work was undertaken in compliance with the guidelines of the Canadian Council on Animal Care.

Isolation and enrichment for epithelial cells

IEC isolation and purification were performed as described previously.[14] Briefly, animals were killed and the small intestine removed, divided into halves, flushed with phosphate buffered saline (PBS), everted, and inflated by injecting PBS. The opposite end was ligated and the segment shaken, using a vortex, in PBS containing 2 mM dithiothreitol (Life Technologies, Burlington, Ontario, Canada). The segments were transferred to a tube containing 30 ml complete medium (RPMI-1640 supplemented with 5% heat inactivated fetal calf serum, 2 mM L-glutamine, 10 mM HEPES, 50 U/ml penicillin, and 50 μg/ml streptomycin; all from Life Technologies) and shaken for 15 second bursts, repeated four times. The cell suspension was centrifuged through 30% Percoll (Pharmacia, Baie d'Urfe, QC) followed by a 45%/75% discontinuous Percoll gradient, each at 550 *g* for 30 minutes. Epithelial cells were collected at the top of the 45% layer and were consistently greater than 90% pure (leucocytes are enriched at the 45%/75% interface).

RNA extraction and relative RT-PCR

Reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously.[2] Briefly, 1 μg of total cellular RNA extracted from each sample using Trizol (Life Technologies) was reverse transcribed using Moloney murine leukaemia virus reverse transcriptase (Life Technologies) with random hexamers as primers. The first strand cDNA was diluted 1:10 for β-actin measurements, otherwise an equal volume was used as template for all cytokine determinations. The PCR mix contained (in final concentrations) 50 mM KCl, 20 mM Tris HCl, pH 8.4, 2.5 mM $MgCl_2$, 0.1 μg/ml bovine serum albumin, 0.2 mM dNTPs, and 2.5 pmol of each primer. Actin, IL-1β, and tumour necrosis factor α (TNF-α) primer sequences are published elsewhere.[14] The sequences used for MIP-2 were: sense 5′GAG CTG CGC TGT CAG TGC CTG3′, antisense 5′AGC CTT GCC TTT GTT CAG TAT3′. PCR was carried out in a Biotherm BioOven III Thermocycler (Bio/Can Scientific) at 93°C for 30 seconds, 60°C for 30 seconds, for 30 or 35 cycles. Amplicons were visualised on 1.5% agarose gels impregnated with 5 μg/ml ethidium bromide and photographed using Polaroid 667 film.

Neutrophil isolation and labelling

Donor neutrophils were isolated from Lewis rats with adjuvant arthritis, as reported elsewhere.[15 16] Briefly, the donor was exchange transfused using 6% hydroxyethylstarch (Hespan; Dupont-Merck Pharmaceuticals, Wilmington, Delaware, USA)/saline with blood collected into acid-citrate dextrose anticoagulant (Formula A; Fenwal-Travenol, Malton, Ontario, Canada). Leucocyte rich plasma was collected after red cell sedimentation (1*g*), centrifuged, and the cell pellet resuspended in Ca^{2+} Mg^{2+} free Tyrode solution containing 5% platelet poor plasma with 3 mg/ml pyrogen free human serum albumin (HSA; Connaught Laboratories, Toronto, Ontario, Canada). The cells were layered on a discontinuous Percoll density gradient (63%/74%) and centrifuged at 400 *g* for 30 minutes at room temperature. Neutrophils, collected from the 63%/74% interface, were consistently greater than 95% viable and pure, and expressed equivalent levels of CD11b/CD18 and L-selectin as neutrophils in whole blood.[17] The purified neutrophils were washed twice in Tyrode solution and labelled with 2 μCi ^{111}In-oxine/10^7 cells (Amersham Corp., Oakville, Ontario, Canada) for 10 minutes at room temperature and then washed again. Under light halothane anaesthesia (Benson Medical Industries Inc., Markham, Ontario, Canada), 5×10^6 neutrophils (labelled with $3–6\times10^5$ cpm) were injected intravenously into the rats.

In some experiments intended to measure the extent of haemorrhage, ^{51}Cr labelled red blood cells (rbc) were injected at the same time as the neutrophils. Following aspiration of the leucocyte rich plasma, 1.0 ml of packed rbc was washed in 10 ml of Tyrode solution and labelled in 3.0 ml Tyrode solution containing 100 μCi/ml $Na_2{}^{51}CrO_4$ (Amersham Corp.). Each rat received 0.2 ml of ^{51}Cr rbc intravenously at the same time as ^{111}In-neutrophils.

Collection of samples and measurement of neutrophil migration

Recipient rats were sacrificed using a CO_2 overdose and whole blood (1 ml) was collected in heparin. The tissue and lumen washout of each organ were recovered separately for gamma counting. The lumen washouts were centrifuged and both the supernatant and pellet (cell associated) counts were determined. Values are reported as cpm per 10^6 cpm injected to represent ^{111}In-neutrophil or ^{51}Cr-rbc accumulation in each site. In addition, a piece of the proximal end of each intestinal segment was fixed in buffered formalin, paraffin embedded, and 5.0 μm sections stained with haematoxylin and eosin.

Adhesion molecule blockade and monoclonal antibodies

The following function blocking mAbs to the rat β_2 integrins were used: anti-CD18 (WT-3, IgG_1),[18] anti-CD11a (TA-3, IgG_1),[19] and anti-CD11b (OX42, IgG_{2a} used as a Fab_2 fragment).[20] Antigen irrelevant mAbs, B9 (IgG_1, anti-Bordetella pertussis toxin)[21] 3H11, or PBS were used as control injections in indomethacin treated animals. Antibodies were administered (1 mg each) immediately prior to injection of labelled cells. In a separate series of experiments, the WT-3 mAb was administered immediately following indomethacin injection.

Statistical analysis

Different treatment groups were compared by non-parametric statistical methods. The Mann-Whitney test was used when there were only two groups. The Kruskal-Wallis test was used for comparison among multiple (>2) groups, and when found to be significant was followed by a post hoc Mann-Whitney test.

RESULTS

Epithelial cell cytokine expression during indomethacin induced intestinal injury

We recovered the small intestinal epithelium from rats and then used Percoll gradients to enrich for epithelial cells (leucocyte depleted) prior to harvesting RNA. This Percoll enrichment typically yielded greater than 90% epithelial cell purity, determined by light microscopic observation of cytocentrifuge preparations. It was noted that in contrast with our experience with helminth infected rats,[3] there were few

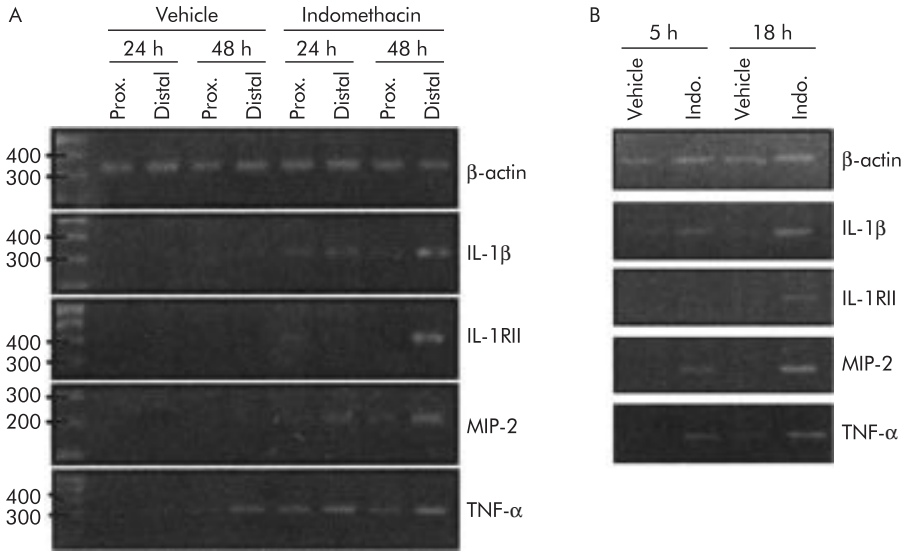

Figure 1 (A) Percoll enriched small intestinal epithelial cells respond to a 15 mg/kg indomethacin injection with inflammatory cytokine expression. At the times indicated, intestinal epithelial cells were isolated from rats given either bicarbonate vehicle or indomethacin subcutaneously, and then total cellular RNA purified and mRNA levels measured using rat specific cytokine primers and reverse transcription-polymerase chain reaction (RT-PCR). (B) Cytokine mRNAs measured by RT-PCR from the epithelium of the distal half the rat small intestine from animals given 15 mg/kg indomethacin (Indo.) and examined at earlier time points than in (A). Using desitometric analysis of the actin bands relative to the cytokine bands, the five hour time point represents as great as 10-fold mRNA levels compared with vehicle levels.[3] IL-1β, interleukin 1β; IL-1RII, interleukin 1 receptor II; MIP-2, monocyte inflammatory peptide 2; TNF-α, tumour necrosis factor α.

neutrophils in the total sloughed epithelial cell preparation from any indomethacin treated rats and therefore virtually none in our Percoll enriched epithelial cells. We previously reported that cytokine mRNAs were detectable from the enriched small intestinal epithelial cell fraction early during the course of infection by *T spiralis*.[2 3] Similarly, the Percoll enriched epithelial cells from 15 mg/kg indomethacin injured small intestine showed amplicons for IL-1β (331 bps), interleukin 1 receptor II (IL-1RII, 382 bp), and MIP-2 (201 bp) (fig 1A). In addition, TNF-α was observed to be present or increased at all time points in indomethacin treated rats compared with control animals. The cytokine mRNAs were detected from both proximal and distal halves of the small intestine but were in greater relative abundance in the distal half at the 15 mg/kg indomethacin dose (fig 1A). Monocyte chemoattractant protein-1 was not detected in the rat epithelium at any of the time points studied in any of the treatment groups (not shown). Another study showed that these cytokine mRNAs were marginally increased at the earliest time point examined, five hours after administration of indomethacin (fig 1B). This array of cytokine expression is consistent with leucocyte recruitment into the small intestine.

Pattern of neutrophil accumulation in the indomethacin injured intestine

In pilot experiments to characterise the kinetics and magnitude of neutrophil infiltration, we allowed ^{111}In labelled neutrophils to circulate and accumulate for 18 hours prior to sacrificing the rats. In those experiments, greater that 90% of the total intestinal ^{111}In was recovered from the lumen (not shown). We therefore reduced the period of labelled cell circulation to three hours in animals at various stages of indomethacin induced inflammation. Figure 2 shows that the site of greatest accumulation in non-inflamed rats was the lumen of the distal half of the small intestine. In indomethacin injured rats, relatively few labelled cells were detected in the stomach and colon, with peak numbers detected at six hours. Neutrophil accumulation was detectable in both halves of the small intestine by six hours, peaked at 24 hours in the proximal half but was highest at 48 hours (the latest time examined) in the distal half (fig 2A). Considering the kinetic data in fig 2, we chose the 24 hour time point and a three hour neutrophil migration period for the adhesion molecule blocking experiments.

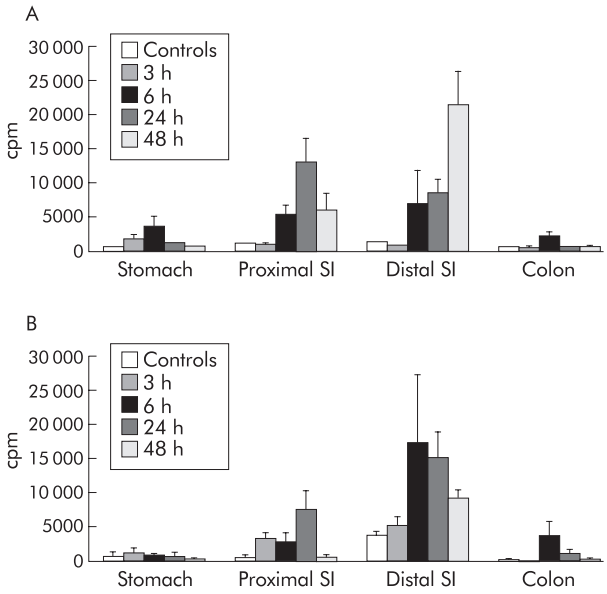

Figure 2 Time course kinetics of neutrophil accumulation in various gastrointestinal tract sites. A single subcutaneous injection of 15 mg/kg indomethacin was given, and then at various times ^{111}In labelled neutrophils were injected and left to circulate for three hours before collecting the tissues and measuring ^{111}In content. (A) Labelled neutrophil accumulation in tissue; (B) accumulation in the lumen. Controls are rats injected with the diluent only. Each time point represents the mean (SEM) of 3–11 rats. SI, small intestine.

CD11/CD18 utilisation by migrating neutrophils

Rats treated with the WT-3 mAb to block CD11/CD18, immediately prior to injecting the labelled neutrophils, showed an 85% reduction in accumulation of ^{111}In neutrophils in the tissue and proximal lumen, and 80% reduction in the

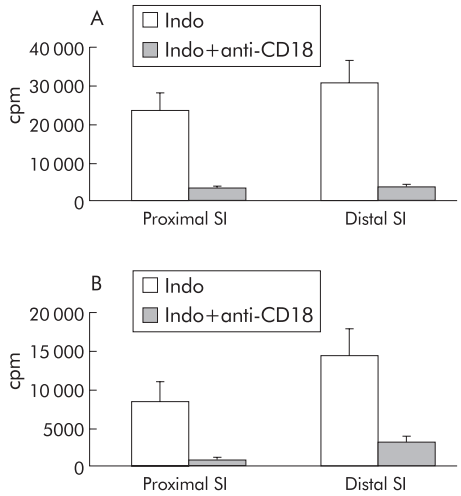

Figure 3 Neutrophil accumulation into the small intestine (SI) of 24 hour indomethacin injected rats is CD18 (β_2 integrin) dependent. (A) Accumulation of labelled cells in the small intestinal tissues of control rats or rats treated with mAb WT-3. (B) Accumulation of labelled cells into the small intestinal lumen of the same rats as in (A). The indomethacin treated control group represents pooled data from rats receiving the control antibody, B9, or phosphate buffered saline injections with the cells (n=7). Data for WT-3 treated rats are pooled from seven animals. All WT-3 treated groups were significantly different (p<0.005) compared with the control group for the same tissue/site.

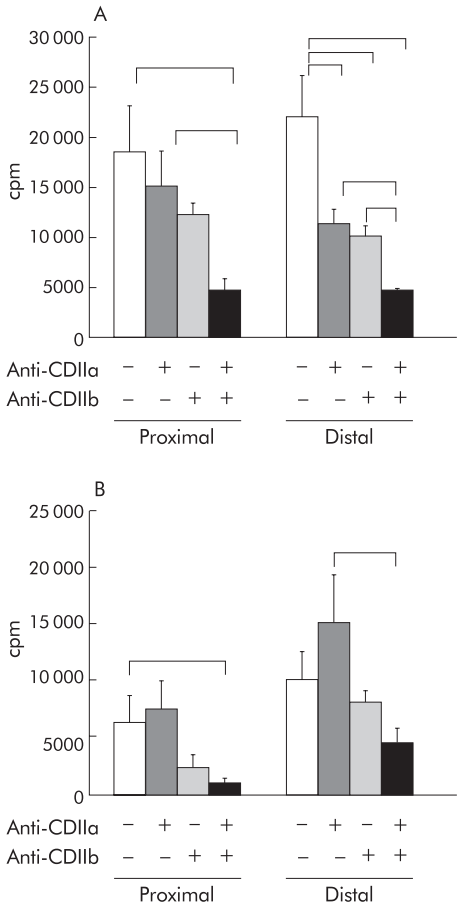

Figure 4 Neutrophil accumulation into the small intestine of 24 hour indomethacin injected rats is CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1) co-dependent. (A) Accumulation of labelled neutrophils in rat small intestinal tissue. (B) Accumulation of labelled neutrophils in the small intestinal lumen. Each bar represents the mean (SEM), with n=7 for the indomethacin treated control group and n=4 for all other groups. Comparisons were made first using the Kruskal-Wallis test and, when found to be significant, comparisons between groups were made using the Mann-Whitney test (p<0.05 indicated by the brackets).

distal lumen (fig 3). Because some haemorrhage with red cell extravasation was observed in the small intestine, we sought to determine the extent of labelled neutrophil accumulation secondary to bleeding. For this we measured ^{51}Cr rbc in the tissue and lumen after three hours—that is, 21–24 hours post indomethacin injection. Based on the accumulation of ^{51}Cr in the intestine, less than 5% of the ^{111}In neutrophil accumulation could be attributed to blood leakage into the intestinal tissue and lumen (not shown).

We then sought to determine which of the CD11/CD18 integrin members neutrophils utilise to infiltrate the intestinal tissue and lumen. Figure 4A shows that mAb to CD11a had a marginal effect on reducing neutrophil accumulation (by 20%) in the proximal half but had a greater (by 50%) and statistically significant effect in the distal small intestine. Anti-CD11b mAb blocked tissue accumulation to a greater extent than anti-CD11a in the proximal half (by 35%) but this did not reach statistical significance. The combination of both antibodies was at least additive and achieved significant differences from control indomethacin treated rats in both halves, similar to the effects of anti-CD18 treatment. Interestingly, the CD11a mAb did not block accumulation in the lumen of the small intestine and even seemed to enhance accumulation in the second half (fig 4B). Blocking CD11b/CD18 resulted in a 63% reduction in neutrophil accumulation in the lumen of the proximal intestine, and a 20% reduction in the distal half. Accumulation in TA-3-treated rats differed statistically from rats receiving the combination of mAbs (fig 4B). The combination of anti-CD11a and CD11b mAbs reduced accumulation to greater than the additive effect of either mAb alone but this reduction is likely to be due to the fact that about 80% of the cells are prevented from even entering the tissues (fig 4A).

The above experiments reveal that CD11a/CD18 and CD11b/CD18 both participate as critical adhesion molecules during neutrophil migration into the small intestine. One possible caveat in the experiments is that accumulation of donor neutrophils is measured, and these neutrophils may not have upregulated CD18 independent adhesion mechanisms. To ascertain whether the unlabelled autologous population of neutrophils was also blocked by the treatments, we injected rats with sufficient anti-CD18 (WT-3 3 mg) at the time of indomethacin injection to block CD18 for 24 hours, then prepared the intestine for histological examination. Figure 5A shows a section of small intestine with a cellular infiltrate underlying an ulcer, prepared from the indomethacin injured rat intestine. Figure 5B shows a cross section of intestine from an animal which received anti-CD18 mAb at the same time as indomethacin; the infiltrate is absent yet the ulcer is present. This result is compatible with the labelled cell data showing that neutrophil migration is CD18 dependent and, further, that ulcer development in this acute injury may be neutrophil independent. This latter point was directly examined by injecting a group of rats with indomethacin, half the group with WT-3, and the remainder with the control mAb, and examining the whole intestine for ulcers 24 hours later. Table 1 reports the number of ulcers in each group of rats after dividing the intestine into quarters, one being the most proximal and four the distal ileum. The most abundant ulcer, which was indistinguishable between the two groups of rats, was typically oval in shape and less than 1 cm in length. However, some long ulcers ran along the mesenteric border for several centimetres. There was no apparent difference in total ulcer surface area between the two groups of rats but there was a trend towards more ulcers in the distal ileum of WT-3-treated

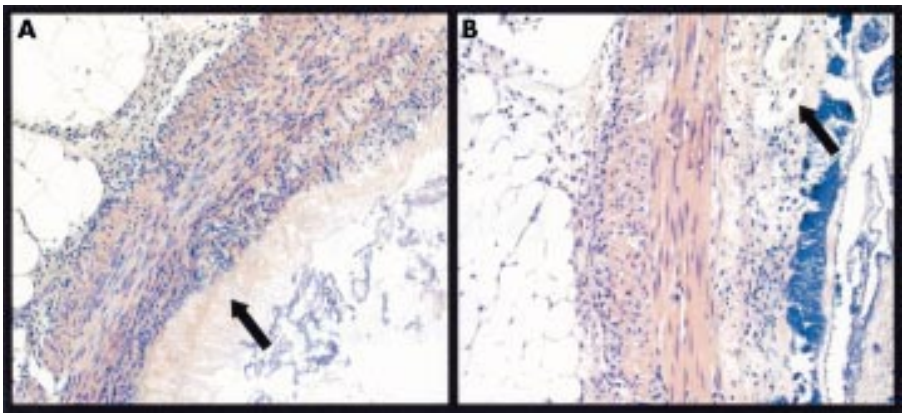

Figure 5 Blockade of CD11/CD18 prevents neutrophil infiltration into the indomethacin injured small intestine. (A) Cross section of jejunum from a rat treated with 15 mg/kg indomethacin 24 hours earlier. (B) Cross section of jejunum of a rat treated with anti-CD18 (WT-3) mAb at the time of indomethacin injection, 24 hours earlier. Both sections show ulcers (arrow) but (B) shows lack of infiltrating cells ("speckling") particularly in the submucosa.

Table 1 Ulcer counts in rats receiving CD18 blocking monoclonal antibody (mAb) or a control antibody at the time of indomethacin injection

	Intestinal length (quarter)			
Treatment (n=3)	1	2	3	4
Control mAb	15*	18 (4)	32 (12)	21 (17)
Anti-CD18 mAb	15 (5)	18 (2)	33 (8)	24 (7)

*One rat had few, but long, ulcers and so only two rats are averaged here.

rats (apparent from the smaller standard deviation). This finding is evidence that neutrophils are unlikely to provoke ulcers in these rats.

DISCUSSION

Epithelial cells are the first cells to encounter intestinal pathogens and toxins. In this role as sentinel cell, after the injury or insult, epithelial cells will secrete signals (cytokines) that recruit leucocytes into the intestine.[22] The indomethacin injured rat small intestine showed a similar array of epithelial inflammatory cytokines as were detected in *T spiralis* infected rats: IL-1β, IL-1RII, and MIP-2, but also TNF-α, which was not found in the helminth infection.[2] Others have reported that the gastric mucosa expresses cytokines, including IL-1α and TNF-α, in response to indomethacin injection.[23] It is noteworthy that some control rats had detectable mRNA for TNF-α in their distal small intestine which seemed to increase with repeated episodes of handling (fig 1A, B). This may be due to stress during the course of the experiment, beginning with injection of vehicle. TNF-α has been demonstrated to play a role in the pathogenesis of the injury due to indomethacin.[9 24 25] TNF shares many biological activities with IL-1, including stimulation of chemokine production and upregulation of endothelial cell adhesion molecules, so while the significance of the difference between the indomethacin and *T spiralis* model is not yet clear, the pattern of cytokines is compatible with initiation of neutrophil recruitment. Whether indomethacin or its metabolites directly or indirectly stimulates cytokine expression by the epithelium was not an objective of these experiments but this non-steroidal anti-inflammatory drug has direct effects on these cells that are worthwhile pursuing as part of the mechanism.[26]

Since the early reports of a possible neutrophil aetiology underlying indomethacin induced gastric ulcers,[8] the topic of migration into gastric tissues has garnered a considerable amount of attention. The small intestinal injury has been less well characterised but neutrophil infiltration nevertheless has been reported by several groups as increased MPO or cell counts from histological preparations.[5 27–29] Our data showing the time course of accumulation in multiple segments of the gastrointestinal tract, and particularly into the lumen, build on these reports. Our kinetics most closely resemble those reported by Nygard and colleagues[5] who showed that the greatest number of cells were detected at 48 hours in rats given 15 mg/kg of indomethacin orally. On the other hand, by including a 24 hour assay point, we showed that neutrophil accumulation peaked at 24 hours in the proximal small intestine and was maximal at 48 hours in the distal half. Yamada and colleagues[30] reported steadily elevated MPO levels over one week in rats injected with a single subcutaneous 7.5 mg/kg dose of indomethacin. Their finding that levels remained elevated and did not peak within a few days may be partially due to the fact that they measured activity in a longitudinal strip representing full length intestine but also as MPO measurements lack cell specificity a late monocyte or eosinophilic infiltration may have contributed to a high MPO signal after the first few days. Indeed, Nygard and colleagues[5] reported that $ED1^+$ macrophages infiltrate the small intestine after 48 hours.

Neutrophil infiltration (transendothelial migration) is a multistep process requiring multiple cell adhesion molecules and cell activation. Experiments from various laboratories have focused on the adhesion molecules relevant for neutrophil migration into the indomethacin injured gastric tissue. For example, one study on the three hour gastric injury due to indomethacin showed that P-selectin blocking antibodies prevented gastric permeability changes although the authors commented that there was little evidence of neutrophil infiltration at this time.[31] Regarding non-gastric infiltration, treatment of rats with anti-E-selectin antibodies proved about half as effective at blocking leucocyte adhesion to, and migration through, mesenteric venules as antibody to CD11b (which did not reduce adherence to control levels), assessed by intravital microscopy.[32] Antibodies to P-selectin reduced rolling and migration but not adherence, and all anti-adhesion molecule treatments reduced MPO in the intestine, suggesting all treatments had some effect on neutrophil migration.[32] Blocking P-selectin in the acetic acid colitis model in rats led to increased superoxide levels without reducing MPO.[33] Thus the roles of the selectins in intestinal disease would appear to be variable and blocking these molecules inconsistently prevents neutrophil migration.

On the other hand, the β_2 integrins are reportedly the principal adhesion molecules used in leucocyte infiltration into the

gut, yet few studies have distinguished which CD11 integrin member—that is, CD11a, b, c, or d—is specifically involved. In one indomethacin report alluded to above, antibodies to CD11b were shown to reduce leucocyte migration in rat mesenteric venules but inhibition was incomplete.[32] Treating TNBS inflamed rats with OX42 resulted in substantially reduced numbers of infiltrating neutrophils but the numbers were not compared with non-inflamed animals and so whether the blockade was absolute was not established.[13] We show here the novel finding that the CD11b/CD18 independent neutrophil accumulation in the indomethacin inflamed intestine is inhibited by mAb to CD11a/CD18 (LFA-1). Furthermore, when the two antibodies are used together the effect is of a similar magnitude as inhibiting the β_2 chain, ruling out any role for CD11c/CD18 or CD11d/CD18. The partial inhibitory effect of each mAb is unlikely to be due to insufficient levels as the doses used maintain at least fivefold saturating levels in the plasma and are proven to inhibit migration in other rat models of leucocyte recruitment.[34] Thus migration of neutrophils into the indomethacin injured small intestine is both CD11a/CD18 and CD11b/CD18 dependent. In order to confirm that this conclusion does not just apply to the injected labelled donor cells, we prepared the intestine from animals 24 hours after injecting the CD18 blocking antibody plus indomethacin. The lack of leucocyte infiltrate is striking in these rats (fig 5B) although the antibody treatment did not prevent ulcers from developing. To affirm that this was not an artefact of our choice of tissue for histological preparation, we counted ulcers in groups of indomethacin treated rats, some with anti-CD18. Table 1 provides unambiguous evidence that ulceration in the distal small bowel is independent of neutrophil infiltration. This result builds on the growing body of evidence, including using neutrophil depletion techniques, showing that neutrophil infiltration may not underlie the development of ulcers.[35 36]

Inhibition of CD11 polypeptides gave clear evidence that either CD11a/CD18 or CD11b/CD18 can serve independently to mediate transendothelial migration but that at least one of these is required. However, CD11a/CD18 does not appear to be required for migration into the lumen as migration was not affected by anti-CD11a mAb treatment (fig 4B). In anti-CD11a treated animals, labelled cells entered the tissue (presumably using CD11b/CD18) and continued seemingly unimpeded or even enhanced into the lumen (also presumably using CD11b). When anti-CD11b mAb was used the effect on blocking migration into the lumen did not reach statistical significance, signifying that an alternative CD11b/CD18 independent mechanism exists, yet it was not obvious that the alternative mechanism was CD11a/CD18. Other integrins, especially the β_1 members VLA-2, VLA-4, VLA-5, VLA-6, and/or VLA-9 may take over when CD18 is blocked as these molecules mediate neutrophil migration across connective tissue barriers.[37 38] Alternatively, CD11a/CD18 and CD11b/CD18 may compete for binding to the same connective tissue ligands with different affinities, and in the absence of one of the CD11 molecules the apparent contribution of the alternate molecule is modified. This was the case in a skin pouch inflammatory model in which ICAM-1 was identified as the counter receptor for CD18, and in which greater numbers of cells migrated in animals deficient in CD11b/CD18, leading the authors to conclude that CD11a/CD18 had a greater affinity for the relevant counter receptors.[39] Based on our data, we conclude that CD11b/CD18 has a higher affinity for connective tissue as blocking CD11a/CD18 led to a slightly greater number of neutrophils migrating into the lumen than in control rats, and blocking CD11b had a greater effect in preventing migration into the lumen.

Our data indicate that neutrophil infiltration in this model is CD18 dependent but CD11a and CD11b co-dependent. We have provided evidence that neutrophils use CD18 independent mechanisms to infiltrate the helminth infected gut but the response to other inflammatory stimuli remains to be determined.[3] It is noteworthy that a patient with leucocyte adhesion deficiency (CD18 deficient) was reported to develop a Crohn's-like ileocolitis.[40] Fully understanding these mechanisms will have implications for developing agents intended to selectively block neutrophil migration into the inflamed intestine.

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Authors' affiliations

A W Stadnyk, Dalhousie Inflammation Group and Department of Pediatrics, and Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada
C Dollard, Dalhousie Inflammation Group and Department of Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada
T B Issekutz, A C Issekutz, Dalhousie Inflammation Group and Departments of Pediatrics, Microbiology and Immunology, and Pathology, Dalhousie University, Halifax, Nova Scotia, Canada

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