

# Anti-inflammatory drugs and endothelial cell adhesion molecule expression in murine vascular beds

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## Abstract

**Background**—Inflammatory bowel diseases (IBD) are characterised by an intense infiltration of leucocytes that is mediated by adhesion molecules expressed on the surface of activated endothelial cells.

**Aims**—To determine whether drugs used in the treatment of IBD, specifically dexamethasone (DEX), 5-aminosalicylic acid (5-ASA), methotrexate (MTX), and 6-mercaptopurine (6-MP), alter the expression of endothelial cell adhesion molecules (ECAMs).

**Methods**—The expression of P-selectin, E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular CAM 1 (VCAM-1) in different vascular beds of C57Bl/6J mice was measured using the dual radiolabelled monoclonal antibody technique.

**Results**—Lipopolysaccharide (LPS) elicited a profound increase in the expression of all ECAMs in the mesentery, small intestine, caecum, and distal colon. The LPS induced increase in CAM expression was not significantly affected by prior treatment with either MTX or 6-MP. However, pretreatment with either DEX or 5-ASA significantly attenuated LPS induced increases in expression of P- and E-selectin, and VCAM-1 in the majority of tissues evaluated. DEX also blunted the LPS induced increase in ICAM-1 expression in the caecum and distal colon. DEX, but not 5-ASA, largely abolished the rise in plasma tumour necrosis factor  $\alpha$  elicited by LPS.

**Conclusions**—These findings suggest that DEX and 5-ASA may exert their beneficial therapeutic action in IBD, at least in part, by inhibiting the expression of ECAMs which mediate leucocyte adhesion and transmigration in the microvasculature.

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**Keywords:** P-selectin; E-selectin; intercellular adhesion molecule 1; vascular cell adhesion molecule 1; dexamethasone; 5-aminosalicylic acid

Inflammatory bowel diseases (IBD) are characterised by an intense infiltration of leucocytes that is associated with an increased expression of adhesion molecules on the surface of activated leucocytes and endothelial cells.<sup>1 2</sup> Data derived from animal models of IBD treated with neutralising antibodies to various

adhesion molecules suggest that there is a cause–effect relation between the expression of endothelial cell adhesion molecules (ECAMs), leucocyte infiltration, and mucosal injury associated with this disease process.<sup>3–5</sup> These findings and results obtained from other models of leucocyte dependent tissue injury have led to an increased interest in defining the factors that regulate the expression of ECAMs. As a consequence, it is now well recognised that leucocyte migration into inflamed tissue results from a cascade of events initiated by the selectin family of adhesion molecules, which mediates the rolling of leucocytes along the endothelium of postcapillary venules.<sup>6–8</sup> A separate group of adhesion glycoproteins (intercellular adhesion molecule 1, ICAM-1; vascular CAM 1, VCAM-1), belonging to the immunoglobulin supergene family, mediate the firm adhesion and transendothelial migration of leucocytes in inflamed microvessels. Studies on cultured endothelial cells<sup>9–11</sup> and using animal models of acute and chronic inflammation have revealed that a number of agents generated or present in inflamed intestine, including cytokines, bacterial endotoxins, and histamine, elicit an increased expression of ECAMs.<sup>12–14</sup> Hence, drugs which inhibit the production or release of these chemical mediators or which directly interfere with the endothelial cell's ability to express adhesion molecules would be predicted to be clinically effective in the treatment of IBD.

Currently, a limited number of compounds have shown clinical efficacy in the treatment of IBD. These include corticosteroids, 5-aminosalicylic acid (5-ASA), methotrexate (MTX) and 6-mercaptopurine (6-MP).<sup>15–17</sup> Studies in animal models of intestinal inflammation have shown that some of these agents can profoundly attenuate the recruitment of leucocytes,<sup>18 19</sup> raising the possibility that these drugs might exert their beneficial effects by inhibiting the expression of ECAMs.

Recently, a novel approach has been developed to quantify the expression of ECAMs in different vascular beds of the rat and mouse.<sup>12–14</sup>

**Abbreviations used in this paper:** 5-ASA, 5-aminosalicylic acid; 6-MP, 6-mercaptopurine; CAM, cell adhesion molecule; DEX, dexamethasone; IBD, inflammatory bowel disease; ICAM, intercellular adhesion molecule; LPS, lipopolysaccharide; MTX, methotrexate; TNF, tumour necrosis factor; VCAM, vascular cell adhesion molecule; ECAM, endothelial cell adhesion molecule; PBS, phosphate buffered saline; BSA, bovine serum albumin; HUVEC, human umbilical vein endothelial cells; IL, interleukin; NF- $\kappa$ B, nuclear factor  $\kappa$ B.

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This technique, based on the use of radiolabelled monoclonal antibodies directed against specific adhesion glycoproteins, has been used in studies designed to define the influence of cytokines and bacterial endotoxin on the expression of P- and E-selectin, ICAM-1, and VCAM-1 in the gastrointestinal vasculature. In the present study, the dual radiolabelled monoclonal antibody technique was used to determine whether drugs that are commonly used for the treatment of IBD—that is corticosteroids, 5-ASA, MTX, and 6-MP, alter the expression of ECAMs elicited by *Salmonella abortus equi*.

## Methods

### MONOCLONAL ANTIBODIES

All the binding monoclonal antibodies used for the in vivo assessment of P-selectin, E-selectin, ICAM-1, and VCAM-1 expression were: RB40.34, a rat IgG1 against mouse P-selectin (PharMingen Inc., San Diego, California, USA)<sup>13 20</sup>; 10E6, a rat IgG2 against mouse E-selectin<sup>13 21</sup>; YN-1, a rat IgG2b directed against mouse ICAM-1 (provided by Bayer Corporation, West Haven, Connecticut, USA)<sup>14 22</sup>; and MK1.9.1, a rat IgG1 targeted against mouse VCAM-1 (provided by Bayer Corporation, West Haven, Connecticut, USA).<sup>14 23 24</sup> P-23, a non-binding murine IgG1 against human P-selectin (Pharmacia-Upjohn, Kalamazoo, Michigan, USA) was also used in the experimental protocols.<sup>25</sup>

### RADIO-IODINATION OF THE MONOCLONAL ANTIBODIES

All the binding monoclonal antibodies, RB40.34, 10E6, YN-1, and MK1.9.1, and the non-binding monoclonal antibody, P-23, were radiolabelled with <sup>125</sup>I and <sup>131</sup>I (Du Pont NEN, Boston, Massachusetts, USA) respectively, using the iodogen method.<sup>26</sup> Briefly, 250 µg of protein was incubated with 250 µCi of sodium <sup>125</sup>I (or sodium <sup>131</sup>I) and 125 µg of iodogen at 4°C for 12 minutes. Phosphate buffered saline (PBS) was added to bring the total volume to 2.5 ml. The radiolabelled monoclonal antibody was then separated from free <sup>125</sup>I by gel filtration on a Sephadex PD-10 column (Pharmacia LKB, Uppsala, Sweden). Phosphate buffer with 1% bovine serum albumin (BSA) was used to equilibrate the column and to elute the radiolabelled antibody. Two 2.5 ml fractions were collected with the second fraction containing the labelled monoclonal antibody. This technique has been previously used for the preparation of both antirat and antimouse ECAM binding monoclonal antibodies.<sup>12–14</sup> Radiolabelled monoclonal antibodies were stored at 4°C. The monoclonal antibodies were dialysed at two week intervals after labelling to ensure minimal free radioactive iodine in the injection. However, less than 1% of the total activity of the protein fraction was recovered after extensive dialysis.

### ANIMALS AND MATERIALS

Male C57Bl/6J mice (n=148) at 8–10 weeks of age were obtained from Harlan Sprague-Dawley, Inc. (Frederick, Maryland, USA) and maintained on normal rodent chow. Lipopolysaccharide (LPS) derived from *Salmonella*

*abortus equi*, and all chemicals used in buffer solutions were obtained from Sigma Chemical Co. (St Louis, Missouri, USA).

### SURGICAL AND EXPERIMENTAL PROCEDURES

The mice were anaesthetised with ketamine hydrochloride (150 mg/kg body weight, intramuscularly) and xylazine (7.5 mg/kg body weight, intramuscularly). The left jugular vein and the right carotid artery were cannulated with polyethylene tubing (PE-10). In order to assess for P- or E-selectin expression, a mixture of 10 µg of either <sup>125</sup>I anti-P-selectin monoclonal antibody (RB40.34) or anti-E-selectin monoclonal antibody (10E6), and an amount of <sup>131</sup>I P-23 necessary to ensure a total <sup>131</sup>I injected activity of 400 000 to 600 000 cpm, was administered through the jugular vein cannula (total volume 200 µl).<sup>13</sup> In the ICAM-1 experiments, a mixture of 6 µg of <sup>125</sup>I anti-ICAM-1 monoclonal antibody (YN-1; 6 µg), and a dose of unlabelled anti-ICAM-1 monoclonal antibody (54 µg) was given with an appropriate amount of <sup>131</sup>I P-23 (400 000 to 600 000 cpm) through the jugular vein cannula (total volume 200 µl).<sup>14</sup> Pilot studies showed that the combination of 6 µg <sup>125</sup>I YN-1 and 54 µg cold YN-1 provided optimum activity to assess accurately ICAM-1 expression and to ensure receptor saturation under constitutive and stimulated conditions. In the VCAM-1 expression experiments, a mixture of 10 µg of <sup>125</sup>I anti-VCAM-1 monoclonal antibody (MK1.9.1), and a dose of unlabelled anti-VCAM-1 monoclonal antibody (10 µg) was given with an appropriate amount of <sup>131</sup>I P-23 (400 000 to 600 000 cpm) through the jugular vein cannula (total volume 200 µl).<sup>14</sup> This dose was selected based on pilot studies showing optimum activity and receptor saturation in the tissues examined under constitutive and stimulated conditions. Administration of cold monoclonal antibody (for ICAM-1 and VCAM-1) allows a pool of radiolabelled monoclonal antibody to remain free in the circulation and available for binding to receptors that are upregulated in response to endothelial cell activation. This is not required for measurement of selectin expression as there is no significant expression of these adhesion molecules under basal conditions and the level of expression under conditions of maximal stimulation is low relative to ICAM-1 and VCAM-1. Previous studies, using blocking doses of the unlabelled monoclonal antibodies, indicate that the <sup>125</sup>I labelled monoclonal antibodies accumulate in tissues as a result of specific ligand binding.<sup>13 14</sup> This conclusion is further supported by experiments showing an absence of binding monoclonal antibody accumulation in mice that are genetically deficient in the respective ECAM (P- or E-selectin or ICAM-1).<sup>13 14</sup>

A sample of blood was obtained from the aortic cannula five minutes after monoclonal antibody injection. Bicarbonate buffered saline was isovolemically exchanged by infusion of buffer through the jugular vein and simultaneous withdrawal of blood/buffer from the aorta following systemic heparinisation (40 units/mouse). The vascular system was then copiously flushed with bicarbonate buffered saline

through the aortic cannula after transection of the inferior vena cava on the pleural aspect of the diaphragm. The following tissues were then harvested and weighed: heart, mesentery, pancreas, stomach, small intestine, caecum, distal colon, and skeletal muscle. Experimental procedures described were performed according to the criteria outlined by the National Institutes of Health and approved by the LSU Medical Centre, Shreveport, committee on animal care and use.

#### CALCULATION OF ECAM EXPRESSION

A 14800 Wizard 3 gamma counter (Wallac, Turku, Finland), with automatic correction for background activity and spillover was used to count  $^{125}\text{I}$  (binding monoclonal antibody) and  $^{131}\text{I}$  (non-binding monoclonal antibody) activities in each organ or tissue and in a 50  $\mu\text{l}$  plasma sample. A 4  $\mu\text{l}$  aliquot of the preinjection mixture of radiolabelled monoclonal antibodies was assayed to determine total injected activity of each labelled monoclonal antibody. The amount of radioactivity remaining in the tube used to mix the monoclonal antibodies, the syringe used to inject the mixture, and jugular vein catheter was subtracted from the total calculated injected activity. ECAM expression was determined by subtracting the accumulated activity of the non-binding monoclonal antibody ( $^{131}\text{I}$  P-23) from that of the binding monoclonal antibody ( $^{125}\text{I}$  RB40.34,  $^{125}\text{I}$  10E6,  $^{125}\text{I}$  YN-1, or  $^{125}\text{I}$  MK1.9.1), and the

data expressed as the percentage of the injected dose (%ID) per gram of tissue. The formula used to calculate ECAM expression was:

$$\text{ECAM expression} = \frac{\% \text{ID} (^{125}\text{I}) \text{g tissue} - \% \text{ID} (^{131}\text{I}) / \text{g tissue} \times \frac{\% \text{ID} (^{125}\text{I}) \text{plasma}}{\% \text{ID} (^{131}\text{I}) \text{plasma}}}{100}$$

This formula was modified from the original method<sup>12</sup> in order to correct for tissue accumulation of the non-binding monoclonal antibody relative to the plasma concentrations of both binding and non-binding monoclonal antibodies.<sup>26</sup> This value, expressed as %ID/g, was converted to  $\mu\text{g}$  monoclonal antibody/g tissue by multiplying the above value by the total injected binding monoclonal antibody ( $\mu\text{g}$ ), divided by 100.

#### EXPERIMENTAL PROTOCOLS FOR ECAM EXPRESSION

ECAM expression was determined under control (constitutive) conditions and at either three hours (for E-selectin), four hours (for P-selectin), or five hours (for ICAM-1 and VCAM-1) following LPS injection (10  $\mu\text{g}/\text{kg}$ , intraperitoneally). The times chosen for determination of induced expression of the different ECAMs represent the periods of peak expression as determined and published in previous reports from our laboratory.<sup>14, 27</sup> In some experiments, either 5-ASA (500  $\text{mg}/\text{kg}$ , orally),

Table 1 Effects of different anti-inflammatory drugs on lipopolysaccharide (LPS) induced P-selectin expression in pancreas, stomach, heart, and skeletal muscle

Treatment	Control	LPS				
		Untreated	5-ASA	DEX	MTX	6-MP
Pancreas	12 (1)	84 (7)	66 (6)	47 (6)*	81 (5)	82 (9)
Stomach	20 (3)	75 (8)	47 (7)	33 (7)*	54 (6)	74 (7)
Heart	7 (1)	94 (11)	56 (7)	56 (7)	74 (10)	111 (13)
Muscle	10 (3)	31 (3)	25 (2)	20 (3)	16 (1)	22 (2)

Results are expressed as mean (SEM) in ng monoclonal antibody/g tissue.

\* $p < 0.05$  v LPS + untreated.

5-ASA, 5-aminosalicylic acid; DEX, dexamethasone; MTX, methotrexate; 6-MP, 6-mercaptopurine.

Table 2 Effects of different anti-inflammatory drugs on lipopolysaccharide (LPS) induced E-selectin expression in pancreas, stomach, heart, and skeletal muscle

Treatment	Control	LPS				
		Untreated	5-ASA	DEX	MTX	6-MP
Pancreas	2 (1)	38 (2)	22 (2)*	20 (2)*	34 (2)	33 (3)
Stomach	3 (1)	36 (1)	23 (3)	25 (2)	36 (4)	38 (5)
Heart	5 (2)	70 (5)	38 (6)*	32 (5)*	79 (10)	86 (8)
Muscle	1 (0)	21 (1)	9 (2)*	7 (0)*	16 (2)	28 (2)

Results are expressed as mean (SEM) in ng monoclonal antibody/g tissue.

\* $p < 0.05$  v LPS + untreated.

5-ASA, 5-aminosalicylic acid; DEX, dexamethasone; MTX, methotrexate; 6-MP, 6-mercaptopurine.

Table 3 Effects of different anti-inflammatory drugs on lipopolysaccharide (LPS) induced intercellular adhesion molecule 1 (ICAM-1) expression in pancreas, stomach, heart, and skeletal muscle

Treatment	Control	LPS				
		Untreated	5-ASA	DEX	MTX	6-MP
Pancreas	543 (14)	1186 (39)	1267 (42)	834 (130)*	1232 (91)	1215 (45)
Stomach	446 (29)	957 (43)	958 (31)	688 (119)	949 (91)	946 (80)
Heart	1256 (71)	3462 (178)	3200 (212)	2147 (507)	3164 (389)	3235 (247)
Muscle	259 (12)	573 (38)	614 (30)	376 (53)	569 (72)	619 (27)

Results are expressed as mean (SEM) in ng monoclonal antibody/g tissue.

\* $p < 0.05$  v LPS + untreated.

5-ASA, 5-aminosalicylic acid; DEX, dexamethasone; MTX, methotrexate; 6-MP, 6-mercaptopurine.

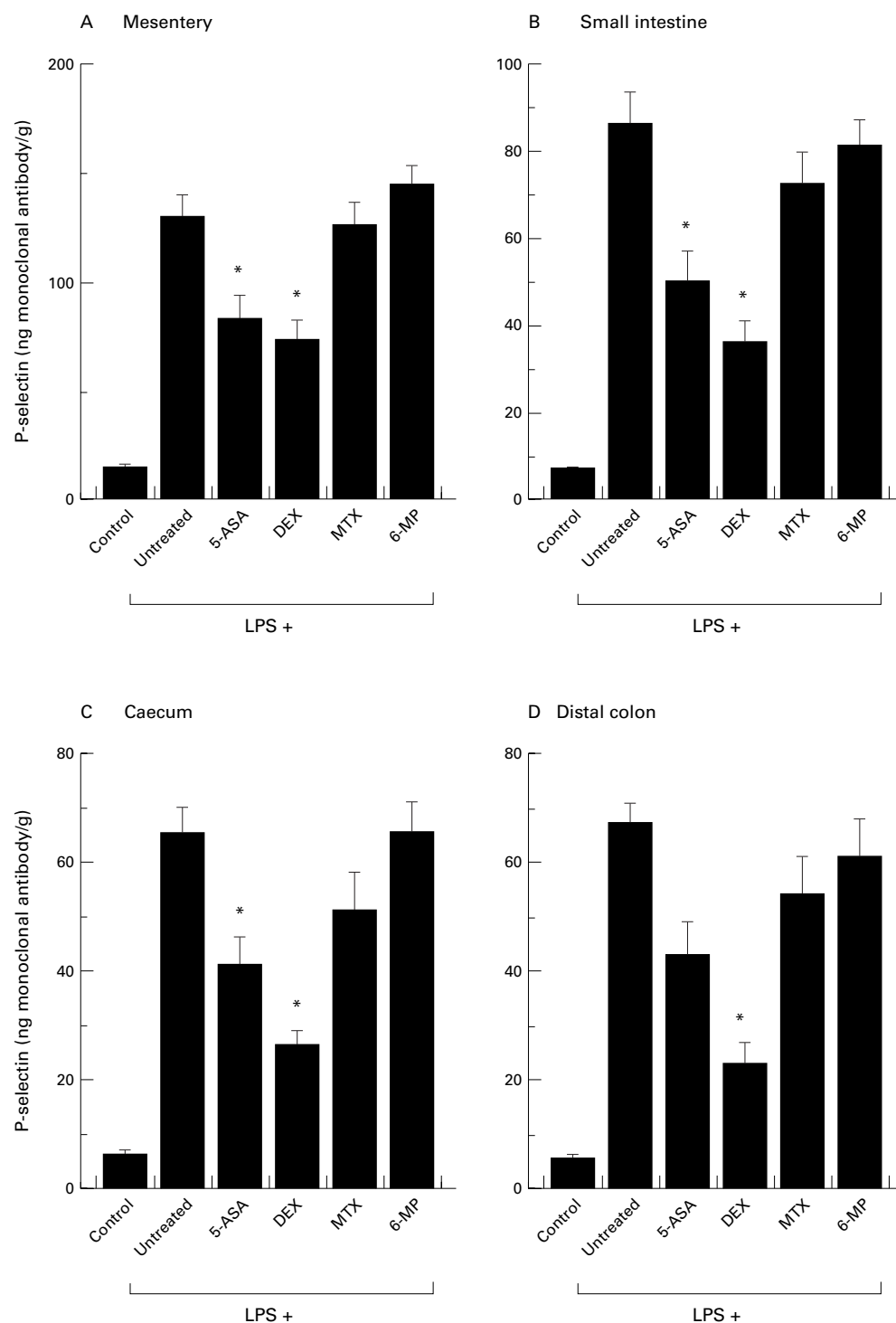


Figure 1 Effect of anti-inflammatory drugs on the expression of P-selectin in (A) mesentery, (B) small intestine, (C) caecum, and (D) distal colon. The numbers of animals in each experimental group was as follows: control, 5; LPS + control, 7; LPS + 5-ASA, 5; LPS + DEX, 5; LPS + MTX, 5; LPS + 6-MP, 5. \* $p < 0.05$  versus LPS + untreated.

MTX (1 mg/kg, orally), or 6-MP (10 mg/kg, orally) were administered 24 two hours prior to injection of LPS. Dexamethasone (DEX, 3 mg/kg, intraperitoneally) was injected two hours prior to injection of LPS. In general, the doses of anti-inflammatory drugs used in this study are about five times the values used to treat human IBD, and are within the range of

doses used to test drug efficacy in rodent models of tissue inflammation.<sup>28-31</sup>

**PLASMA TUMOUR NECROSIS FACTOR  $\alpha$  BIOASSAY**  
Plasma tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) concentrations were measured in samples obtained from the inferior vena cava of

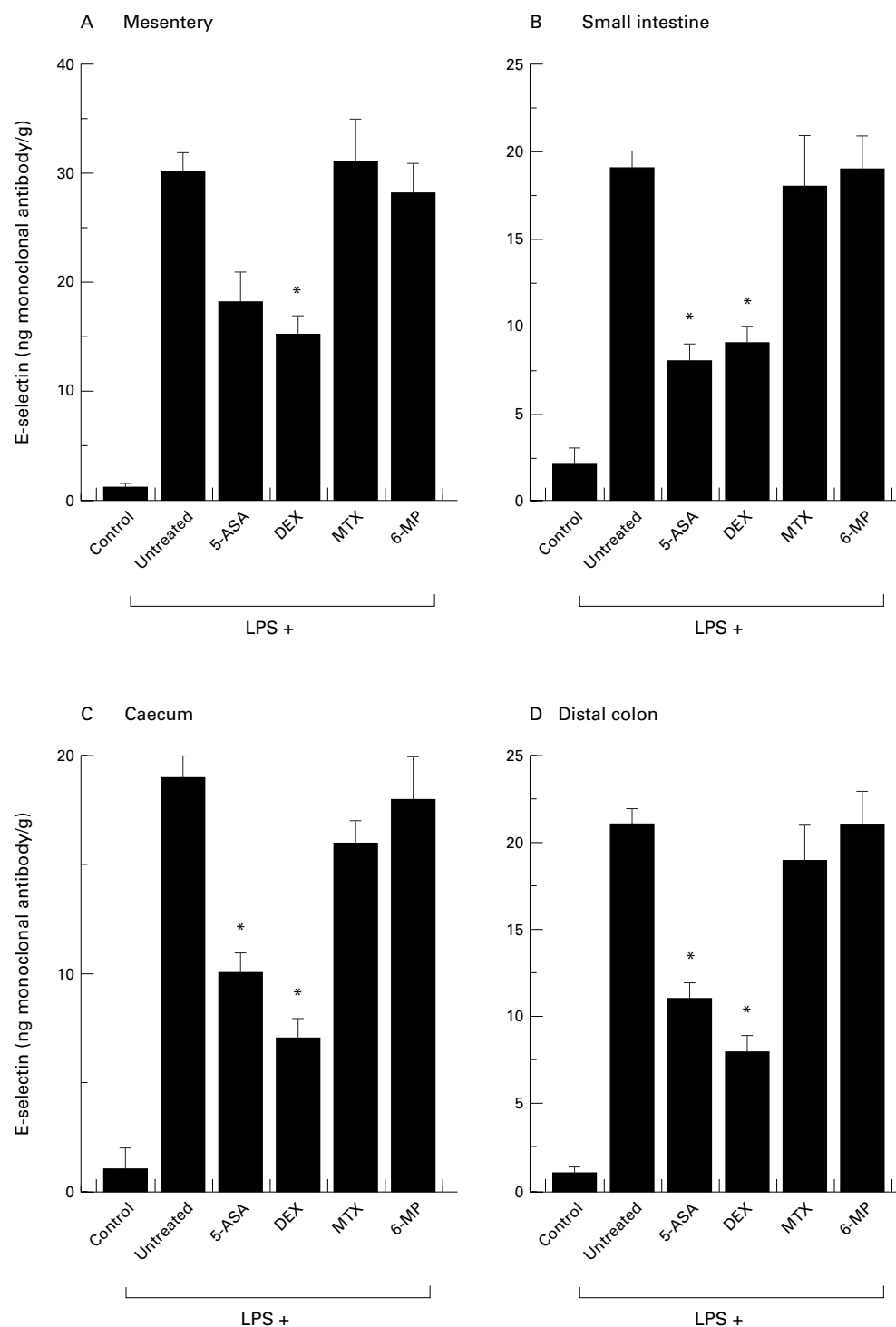


Figure 2 Effect of anti-inflammatory drugs on the expression of E-selectin in (A) mesentery, (B) small intestine, (C) caecum, and (D) distal colon. The numbers of animals in each experimental group were as follows: control, 5; LPS + control, 7; LPS + 5-ASA, 5; LPS + DEX, 5; LPS + MTX, 5; LPS + 6-MP, 5. \* $p < 0.05$  versus LPS + untreated.

non-stimulated (constitutive) and LPS stimulated animals. LPS was administered (10  $\mu$ g/kg, intraperitoneally) one hour prior to obtaining plasma samples that were used to assay for TNF- $\alpha$  with a commercially available enzyme linked immunosorbent assay (ELISA) kit for murine TNF- $\alpha$  (BioSource International Inc., Camarillo, California, USA).

#### STATISTICAL ANALYSES

All data were analysed using standard statistical analyses—that is, one way analysis of variance (ANOVA) and Scheffe's (post hoc) test. A one sample sign test (StatView 4.02 for Macintosh computers) was used to determine whether the value of ECAM expression was significantly different from zero. Values determined not to be

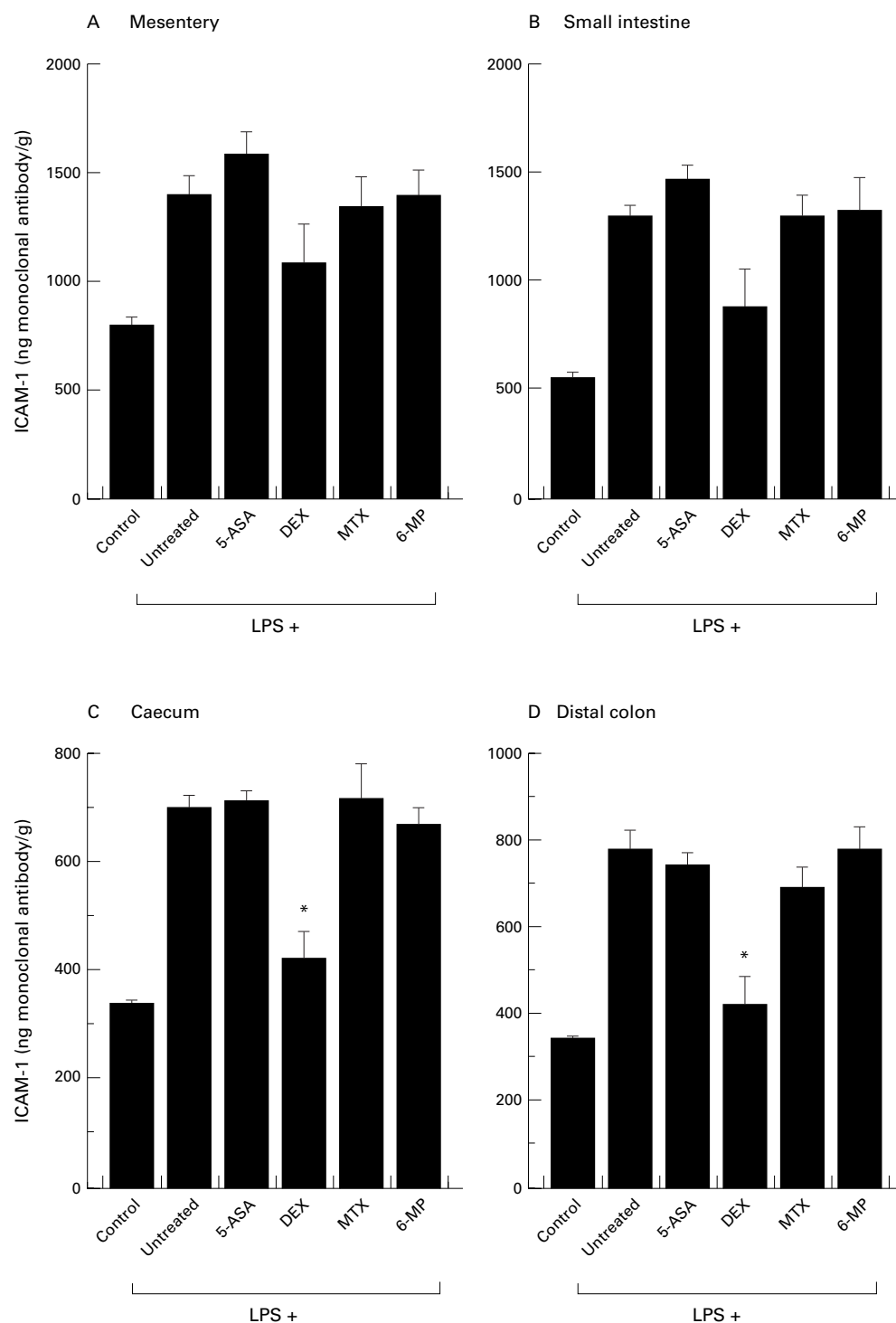


Figure 3 Effect of anti-inflammatory drugs on the expression of ICAM-1 in (A) mesentery, (B) small intestine, (C) caecum, and (D) distal colon. The numbers of animals in each experimental group were as follows: control, 5; LPS + control, 7; LPS + 5-ASA, 5; LPS + DEX, 5; LPS + MTX, 5; LPS + 6-MP, 5. \* $p < 0.05$  versus LPS + untreated.

different from zero are presented as zero in the text. All other values are reported as mean (SEM), with five to seven mice per group. Statistical significance was set at  $p < 0.05$ .

## Results

### P-SELECTIN EXPRESSION

Figure 1 compares the effect of treatment with the different anti-inflammatory drugs on the expression of P-selectin in the mesentery, small

intestine, caecum, and distal colon. Table 1 summarises P-selectin expression in other organs. LPS elicited an increased expression of P-selectin, as measured by the specific accumulation of  $^{125}\text{I}$  RB40.34, in all organs. 5-ASA attenuated the endotoxin induced P-selectin expression in the mesentery, small intestine, and caecum. DEX blunted the LPS induced expression of P-selectin in all organs studied except heart and muscle. The inhibitory action



Table 4 Effects of different anti-inflammatory drugs on lipopolysaccharide (LPS) induced vascular cell adhesion molecule 1 (VCAM-1) expression in pancreas, stomach, heart, and skeletal muscle

Treatment	Control	LPS				
		Untreated	5-ASA	DEX	MTX	6-MP
Pancreas	66 (4)	223 (6)	161 (7)*	150 (6)*	169 (5)*	205 (13)
Stomach	93 (10)	309 (16)	256 (13)	193 (3)*	255 (14)	310 (23)
Heart	117 (12)	761 (41)	602 (29)*	584 (37)*	558 (39)*	673 (52)
Muscle	34 (3)	112 (7)	68 (7)*	56 (6)*	90 (6)	105 (5)

Results are expressed as mean (SEM) in ng monoclonal antibody/g tissue.

\* $p < 0.05$  v LPS + untreated.

5-ASA, 5-aminosalicylic acid; DEX, dexamethasone; MTX, methotrexate; 6-MP, 6-mercaptopurine.

of DEX on P-selectin expression was consistently greater than that observed with 5-ASA. Neither MTX nor 6-MP had a significant influence on the LPS induced increase in P-selectin expression in any organ.

#### E-SELECTIN EXPRESSION

Intraperitoneal injection of LPS elicited a profound increase in E-selectin expression in all tissues studied (fig 2, table 2). In a manner similar to that noted for P-selectin, LPS induced expression of E-selectin was significantly attenuated in almost all organs by pretreatment with either 5-ASA or DEX. MTX and 6-MP had no effect on LPS mediated E-selectin expression.

#### ICAM-1 EXPRESSION

ICAM-1 was found to be constitutively expressed in all organs studied. The increment in ICAM-1 expression elicited by LPS was smaller than that observed with the selectins. 5-ASA, MTX, and 6-MP had no effect on ICAM-1 expression in any tissue studied. DEX reduced ICAM-1 expression but only in the caecum and distal colon (fig 3, table 3); however, reductions (non-significant) were noted for mesentery and small intestine.

#### VCAM-1 EXPRESSION

The constitutive level of VCAM-1 expression in all tissues studied was much lower than the value obtained for ICAM-1. Consequently, LPS elicited a more robust upregulation of VCAM-1 in all vascular beds (fig 4, table 4). 5-ASA was effective at reducing LPS induced VCAM-1 expression in all organs except stomach and mesentery. DEX, on the other hand, significantly blunted VCAM-1 expression in all organs, and the extent of the inhibition tended to be greater than observed with 5-ASA. Neither MTX nor 6-MP exerted a significant effect on VCAM-1 expression in any tissue studied.

#### PLASMA TNF- $\alpha$ CONCENTRATIONS

Figure 5 illustrates the changes in plasma TNF- $\alpha$  concentrations noted after LPS administration in untreated mice and in mice receiving either 5-ASA or DEX. LPS notably increased plasma TNF- $\alpha$  concentrations. Although 5-ASA did not attenuate the LPS induced increase in plasma TNF- $\alpha$ , DEX completely abolished the TNF- $\alpha$  response.

#### Discussion

Although the aetiology of inflammatory bowel diseases remains poorly understood, there is

growing recognition that an imbalance of the intestinal immune system, possibly elicited by products of enteric bacteria, probably contributes to this disease process.<sup>32</sup> The resulting emphasis on immune dysfunction has prompted a search for new therapeutic agents that target specific components of the immune system. In addition, there is an increasing effort to reexamine drugs that are now routinely used in the treatment of IBD for potential actions that restore immune function in IBD. Molecules that regulate the recruitment of leucocytes such as ECAMs have been a recent focus of this attention. This attention can be justified on the basis of several studies that have shown blunted leucocyte recruitment and tissue injury responses in animal models of IBD after administration of blocking antibodies against ECAMs.<sup>3-5</sup> The results of the present study extend these observations by providing evidence that implicates a role for suppression of ECAM expression in the therapeutic action of corticosteroids and 5-ASA, two drugs used in treatment of IBD.

Overall, the findings of the present study indicate that both 5-ASA and dexamethasone are effective in reducing the upregulation in ECAM expression elicited by LPS in several tissues. These effects are noted at concentrations that are within five times the doses used therapeutically<sup>15 33 34</sup> and both drugs affected all of the ECAMs studied (P-selectin, E-selectin, ICAM-1, and VCAM-1). The actions of dexamethasone were exerted in a more widespread fashion, with a larger number of vascular beds responding to corticosteroid treatment. Furthermore, the inhibitory actions of dexamethasone appeared to be most profound in distal colon and caecum. In contrast to the inhibitory actions of 5-ASA and dexamethasone, the immunosuppressive agents methotrexate and 6-mercaptopurine had no significant effect on the LPS induced expression of all four ECAMs.

Corticosteroids have been successfully used to treat moderate to severe forms of IBD since the 1950s. Although the mode of action of corticosteroids remains undefined, there is a growing body of evidence in the literature that implicates leucocyte-endothelial cell adhesion as a principal target for these compounds. Both in vitro<sup>35 36</sup> and in vivo<sup>37 38</sup> models of leucocyte-endothelial cell adhesion have revealed an antiadhesive effect of corticosteroid treatment. For example, a recent report by Taylor and colleagues<sup>39</sup> describes an attenuating effect (about a 50% reduction) of dexam-

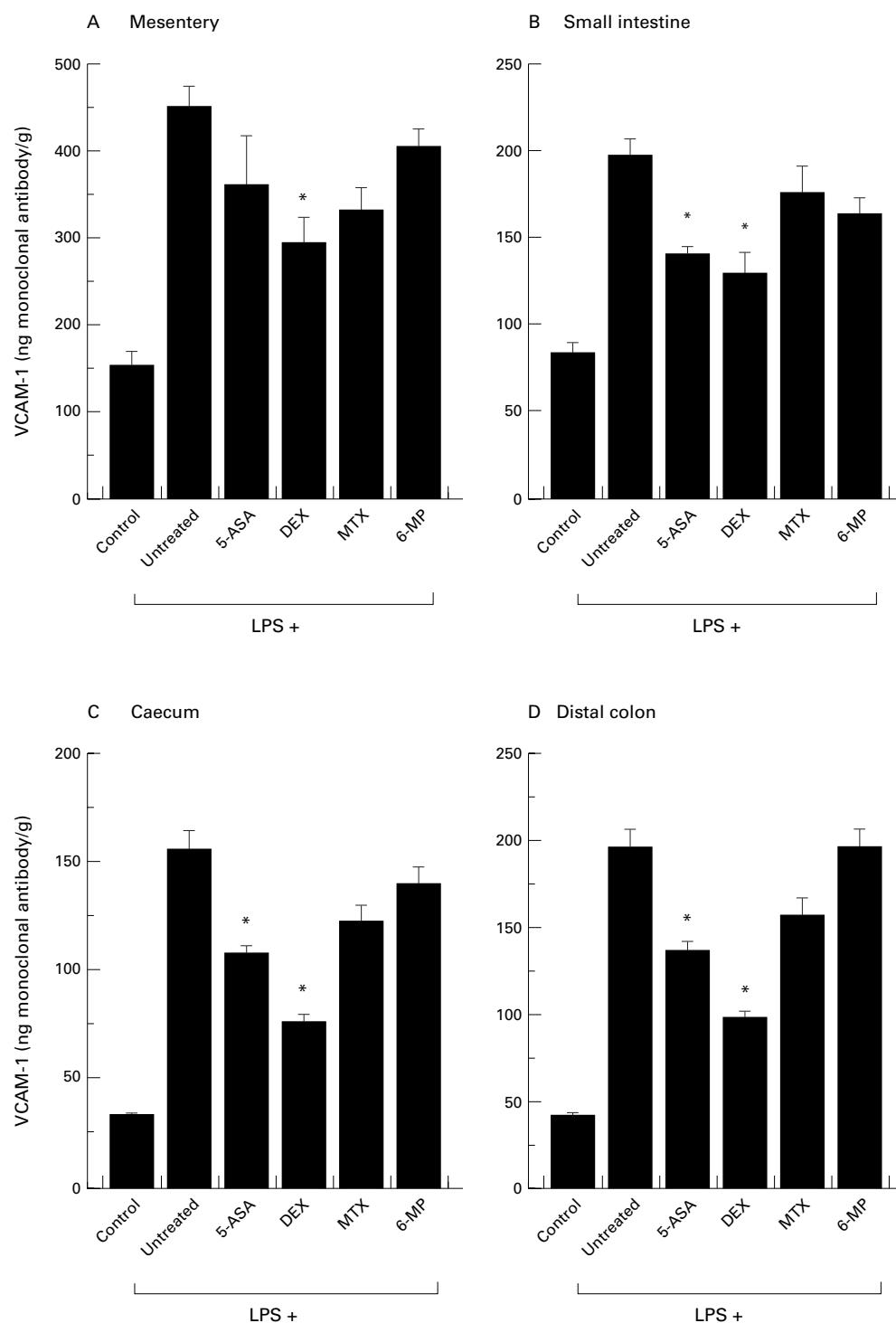


Figure 4 Effect of anti-inflammatory drugs on the expression of VCAM-1 in (A) mesentery, (B) small intestine, (C) caecum, and (D) distal colon. The numbers of animals in each experimental group were as follows: control, 5; LPS + control, 7; LPS + 5-ASA, 5; LPS + DEX, 5; LPS + MTX, 5; LPS + 6-MP, 5. \* $p < 0.05$  versus LPS + untreated.

ethasone pretreatment on leucocyte adherence and a more profound (more than 90%) effect on leucocyte emigration in rat mesenteric venules stimulated with interleukin 1 $\beta$ . These observations are consistent with our findings that dexamethasone significantly blunts the induced expression of ECAMs that are involved in leucocyte rolling (selectins), adherence (ICAM-1, VCAM-1), and emigration (ICAM-1, VCAM-1).

Monolayers of cultured human umbilical vein endothelial cells (HUVEC) have yielded conflicting results concerning the effect of corticosteroids on CAM expression. Cronstein *et al* have reported that dexamethasone inhibits endotoxin induced expression of ICAM-1 and E-selectin on HUVEC.<sup>35</sup> A similar inhibitory action of dexamethasone on VCAM-1, ICAM-1, and E-selectin expression has been noted using either TNF or interleukin 1 (IL-1)



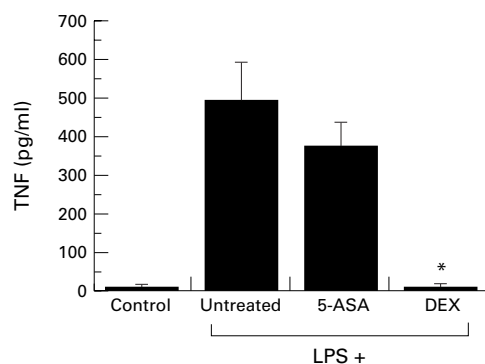


Figure 5 Effect of 5-ASA and DEX on plasma TNF- $\alpha$  concentrations in stimulated animals. The numbers of animals in each experimental group was as follows: control, 5; LPS + control, 5; LPS + 5-ASA, 5; LPS + DEX, 5. \* $p < 0.05$  versus LPS + untreated.

activated HUVEC.<sup>36</sup> Some cultured endothelial cells have also revealed that dexamethasone decreases cytokine induced E-selectin expression by inhibiting the activation of the nuclear transcription factor NF- $\kappa$ B,<sup>40</sup> which is found in the promoter region of the genes encoding for VCAM-1 and ICAM-1, as well as E-selectin.<sup>41</sup> There are also reports which indicate that corticosteroids exert no effect on cytokine induced expression of ICAM-1.<sup>42, 43</sup>

The results of our study and data already in the literature<sup>38</sup> indicate that dexamethasone may exert some of its *in vivo* actions on LPS induced ECAM expression by blunting the production and/or release of cytokines normally elicited *in vivo* by LPS administration. We noted that dexamethasone, but not 5-ASA, completely prevented the elevation in plasma TNF- $\alpha$  induced in mice receiving LPS. This observation is consistent with previous reports that describe an attenuating influence of dexamethasone on the LPS induced generation of proinflammatory cytokines such as IL-1, TNF- $\alpha$ , and interferon  $\gamma$ .<sup>38</sup> This however may not be the sole mechanism of action of dexamethasone since we have also observed (unpublished observation) that dexamethasone blocks the increased expression of ECAMs that is elicited by TNF- $\alpha$  per se.

5-ASA is the therapeutically active moiety of sulphasalazine, which is one of the most widely used drugs for the treatment of IBD.<sup>16, 17</sup> Several mechanisms, including free radical scavenging, inhibition of leukotriene production, and diminished cytokine generation, have been invoked to explain the anti-inflammatory actions of 5-ASA.<sup>44</sup> Studies in animal models of IBD have revealed that 5-ASA pretreatment significantly blunts the infiltration of leucocytes into the inflamed intestinal mucosa.<sup>18, 19</sup> The results of our study suggest that at least part of the beneficial actions of 5-ASA may be related to the ability of this drug to inhibit the expression of ECAMs. Although 5-ASA was not as uniformly effective as dexamethasone in reducing the LPS induced upregulation of ECAMs, the aminosalicilate did exert a dramatic influence on the expression of E-selectin in distal bowel segments (caecum and distal colon). This response, coupled to its weaker action on P-selectin expression, could

result in a dramatic reduction in the number of rolling leucocytes in postcapillary venules of the inflamed bowel. The corresponding action of 5-ASA on VCAM-1 expression in the distal bowel could also result in an attenuated recruitment of firmly adherent leucocytes, particularly monocytes and lymphocytes which utilise VCAM-1 for adherence to and emigration across vascular endothelium.<sup>5, 11</sup>

Although 5-ASA did not affect the LPS induced generation of plasma TNF- $\alpha$ , the drug may blunt ECAM expression by modulating the production of other cytokines, such as IL-1.<sup>44</sup> 5-ASA has also been reported to reduce the production of prostaglandins, thromboxanes, and platelet activating factor, to inhibit the release of histamine from mast cells, and to scavenge oxygen free radicals.<sup>44, 45</sup> Hence, it is possible that one or more of these actions ultimately accounts for the modulating influence of 5-ASA on ECAM expression *in vivo*. Inasmuch as NF- $\kappa$ B activation can be elicited by oxygen radicals,<sup>46</sup> it is conceivable that 5-ASA interferes with an LPS induced oxidant stress in endothelial cells, prevents the activation of NF- $\kappa$ B, and consequently blunts the upregulation of ECAMs.

Methotrexate has been previously shown to inhibit the adhesion of leucocytes to vascular endothelium.<sup>47, 48</sup> This action appears to result from the increased production of adenosine, which consequently inhibits leucocyte-endothelial cell adhesion through occupancy of adenosine A2 receptors on leucocytes.<sup>47, 48</sup> This action of methotrexate occurs rapidly (within minutes), affects primarily the leucocyte, and does not appear to involve changes in the transcriptional regulation of ECAMs. Hence, the absence of an effect of methotrexate and 6-mercaptopurine on LPS induced ECAM expression is not inconsistent with the observation that these agents can blunt the accumulation of leucocytes in inflamed tissue. Additional studies are needed, however, to assess definitively whether chronic treatment with either methotrexate or 6-mercaptopurine alters ECAM expression, and whether such treatment with either dexamethasone or 5-ASA would result in more profound attenuations in ECAM expression.

While the findings of this study show profound reductions in ECAM expression after treatment with certain agents used to treat IBD, it remains unclear whether and to what extent leucocyte trafficking is affected by these reductions in ECAM expression. Previous studies have shown that the constitutive level of expression of ICAM-1 in the intestinal vasculature is sufficient to recruit large numbers of adherent and emigrating leucocytes in postcapillary venules.<sup>6</sup> Hence, an appreciation of the pathological significance of the decline in ECAM expression elicited by dexamethasone and 5-ASA must await studies that specifically examine the quantitative relation between ECAM expression and leucocyte recruitment.

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- 1 Malizia G, Calabrese A, Cottone M, *et al.* Expression of leukocyte adhesion molecules by mucosal mononuclear phagocytes in inflammatory bowel disease. *Gastroenterology* 1991;100:150–9.
- 2 Koizumi M, King N, Lobb R, *et al.* Expression of vascular adhesion molecules in inflammatory bowel disease. *Gastroenterology* 1992;103:840–7.
- 3 Picarella D, Hurlbut P, Rottman J, *et al.* Monoclonal antibodies specific for beta 7 integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) reduce inflammation in the colon of SCID mice reconstituted with CD45RB<sup>high</sup> CD4+ T cells. *J Immunol* 1997;158:2099–106.
- 4 Podolsky DK, Lobb R, King N, *et al.* Attenuation of colitis in the cotton-top tamarin by anti- $\alpha 4$  integrin monoclonal antibody. *J Clin Invest* 1993;92:372–80.
- 5 Panés J, Granger DN. Leukocyte-endothelial cell interactions: molecular mechanisms and implications in gastrointestinal disease. *Gastroenterology* 1998;114:1066–90.
- 6 Granger DN, Kubes P. The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion. *J Leuk Biol* 1994;55:662–75.
- 7 Kubes P, Kanwar S. Histamine induces leukocyte rolling in postcapillary venules: a P-selectin-mediated event. *J Immunol* 1994;152:3570–7.
- 8 Lawrence MB, Springer TA. Neutrophils roll on E-selectin. *J Immunol* 1993;151:6338–46.
- 9 Shen J, Ham RG, Karmiol S. Expression of adhesion molecules in cultured human pulmonary microvascular endothelial cells. *Microvasc Res* 1995;50:360–72.
- 10 Haraldsen G, Kvale D, Lein B, *et al.* Cytokine-regulated expression of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in human intestinal microvascular endothelial cells. *J Immunol* 1996;156:2558–65.
- 11 Hensleit U, Steinbrink K, Sunderkotter C, *et al.* Expression of murine VCAM-1 in vitro and in different models of inflammation in vivo: correlation with emigration of monocytes. *Exp Dermatol* 1994;3:249–56.
- 12 Panés J, Perry MA, Anderson DC, *et al.* Regional differences in constitutive and induced ICAM-1 expression in vivo. *Am J Physiol* 1995;269:H1955–64.
- 13 Eppihimer MJ, Wolitzky B, Anderson DC, *et al.* Heterogeneity of expression of E- and P-selectins in vivo. *Circ Res* 1996;79:560–9.
- 14 Henninger DD, Panés J, Eppihimer M, *et al.* Cytokine-induced VCAM-1 and ICAM-1 expression in different organs of the mouse. *J Immunol* 1997;158:1825–32.
- 15 Sutherland LR, Collins SM, Martin F, *et al.* *Inflammatory bowel disease: basic research, clinical implications and trends in therapy.* Lancaster, UK: Kluwer Academic Publishers, 1994.
- 16 Bonner FB, Lauderdale F. Current medical therapy for inflammatory bowel disease. *South Med J* 1996;89:556–66.
- 17 Stotland BR, Lichtenstein GR. Newer treatments for inflammatory bowel disease. *Prim Care Med* 1996;23:577–608.
- 18 Fitzpatrick LR, Bostwick JS, Renzetti M, *et al.* Anti-inflammatory effects of various drugs on acetic acid induced colitis in the rat. *Agents Actions* 1990;30:393–402.
- 19 Noronha-Blob L, Lowe VC, Muhlhauser RO, *et al.* NPC 15669, an inhibitor of neutrophil recruitment, is efficacious in acetic acid-induced colitis in rats. *Gastroenterology* 1993;104:1021–9.
- 20 Patel KD, Moore KL, Nollert MU, *et al.* Neutrophils use both shared and distinct mechanisms to adhere to selectin. *J Clin Invest* 1995;96:1887–96.
- 21 Norton CR, Rumberger JM, Burns DK, *et al.* Characterization of murine E-selectin expression in vivo using novel anti-mouse E-selectin monoclonal antibodies. *Biochem Biophys Res Commun* 1993;195:250–8.
- 22 Takei F. Inhibition of mixed lymphocyte response by a rat monoclonal antibody to a novel murine lymphocyte activation antigen (MALA-2). *J Immunol* 1985;134:1403–7.
- 23 Miyake K, Weissman IL, Greenberger JS, *et al.* Evidence for a role of the integrin VLA-4 in lymphohemopoiesis. *J Exp Med* 1991;173:599–607.
- 24 Miyake K, Medina K, Ishihara K, *et al.* A VCAM-like adhesion molecule on murine bone marrow stromal cells mediates binding of lymphocytes precursor in culture. *J Cell Biol* 1991;114:557–65.
- 25 Ma L, Raycroft L, Asa D, *et al.* A sialoglycoprotein from human leukocytes functions as a ligand for P-selectin. *J Biol Chem* 1994;269:27739–46.
- 26 Fraker PJ, Speck JC. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. *Biochem Biophys Res Commun* 1978;80:849–57.
- 27 Eppihimer MJ, Russell J M, Anderson DC, *et al.* Endothelial cell adhesion molecule expression in gene-targeted mice. *Am J Physiol* 1997;273:H1903–8.
- 28 Kojouharoff G, Hans W, Obermeier F, *et al.* Neutralization of tumour necrosis factor but not IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. *Clin Exp Immunol* 1997;107:353–8.
- 29 Zijlstra FJ, Garredts IM, van Dijk AP, *et al.* Experimental colitis in mice: effects of olsalazine on eicosanoid production in colonic tissue. *Agents Actions* 1992; Spec No:C76–C78.
- 30 Karl PI, Katz R, Daum F, Fisher SE. 6-Mercaptopurine and spermatogenesis in the young rat. *Dig Dis Sci* 1991;36:1596–73.
- 31 DeJoy SQ, Jeyaseelan R, Torley LW, *et al.* Attenuation of interleukin 2-induced pulmonary vascular leak syndrome by low doses of oral methotrexate. *Cancer Res* 1995;55:4929–35.
- 32 James SP, Klapproth JM. Major pathway of mucosal immunity and inflammation: cell activation, cytokine production and the role of bacterial factors. *Aliment Pharmacol Ther* 1996;10(suppl 2):1–9.
- 33 Hibi T, Iwao Y, Yajima T, *et al.* Immunosuppressive agents in the treatment of Crohn's disease and ulcerative colitis. *J Gastroenterol* 1995;30:121–3.
- 34 Denniston PL, Edwards KM, LaBash JJ, *et al.* *Physicians GenRx* 1995. Riverside, CT: Denniston Publishing Inc., 1995.
- 35 Cronstein BN, Kimmel SC, Levin RL, *et al.* A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocytes adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. *Proc Natl Acad Sci USA* 1992;89:9991–5.
- 36 Aziz KE, Wakefield D. Modulation of endothelial cell expression of ICAM-1, E-selectin, and VCAM-1 by  $\beta$ -estradiol, progesterone, and dexamethasone. *Cell Immunol* 1996;167:79–85.
- 37 McCafferty DJ, Granger DN, Wallace JL. Indomethacin-induced gastric injury and leukocyte adherence in arthritic versus healthy rats. *Gastroenterology* 1995;109:1173–80.
- 38 Klemm P, Harris HJ, Perretti M. Effect of rolapram in a murine model of acute inflammation: comparison with the corticoid dexamethasone. *Eur J Pharmacol* 1995;281:69–74.
- 39 Taylor A, Flower RJ, Perretti M. Dexamethasone inhibits leukocyte emigration in rat mesenteric post-capillary venules: an intravital microscopy study. *J Leuk Biol* 1997;62:301–8.
- 40 Brostjan C, Anrather J, Csizmadia V, *et al.* Glucocorticoids inhibit E-selectin expression by targeting NF- $\kappa$ B and not ATF/c-Jun. *J Immunol* 1997;158:3836–44.
- 41 Collins T. Endothelial nuclear factor- $\kappa$ B and the initiation of the atherosclerotic lesion. *Lab Invest* 1993;68:499–508.
- 42 Detmar M, Tenorio S, Hettmannsperger U, *et al.* Cytokine regulation of proliferation and ICAM-1 expression of human dermal microvascular endothelial cells in vitro. *J Invest Dermatol* 1992;98:147–53.
- 43 Hettmannsperger U, Tenorio S, Orfanos CE, *et al.* Corticosteroids induce proliferation but do not influence TNF or IL-1 beta induced ICAM-1 expression of human dermal microvascular cells in vitro. *Arch Dermatol Res* 1993;285:347–51.
- 44 Greenfield SM, Punchard NA, Teare JP, *et al.* Review article: the mode of action of the aminosaliclates in inflammatory bowel disease. *Aliment Pharmacol Ther* 1993;7:369–83.
- 45 Travis SP, Jewell DP. Salicylates for inflammatory bowel disease. *Baillieres Clin Gastroenterol* 1994;8:203–31.
- 46 Meyer M, Schreck R, Baueerle PA. H<sub>2</sub>O<sub>2</sub> and antioxidants have opposite effects on activation of NF- $\kappa$ B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J* 1993;12:2005–15.
- 47 Asako H, Kubes P, Baethge BA, *et al.* Colchicine and methotrexate reduce leukocyte adherence and emigration in rat mesenteric venules. *Inflammation* 1992;16:45–56.
- 48 Cronstein BN, Naime D, Ostad E. The anti-inflammatory mechanism of methotrexate. *J Clin Invest* 1993;92:2675–82.