

Dexamethasone inhibition of leucocyte adhesion to rat mesenteric postcapillary venules: role of intercellular adhesion molecule 1 and KC

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Abstract

Background—A previous study showed that the glucocorticoid dexamethasone, at doses of 100 µg/kg and above, inhibited leucocyte adhesion to rat mesenteric postcapillary venules activated with interleukin 1β (IL-1β), as assessed by videomicroscopy.

Aims—To identify whether the adhesion molecule, intercellular adhesion molecule 1 (ICAM-1), or the chemokine KC could be targeted by the steroid to mediate its antiadhesive effect.

Methods—Rat mesenteries were treated with IL-1β (20 ng intraperitoneally) and the extent of leucocyte adhesion measured at two and four hours using intravital microscopy. Rats were treated with dexamethasone, and passively immunised against ICAM-1 or KC. Endogenous expression of these two mediators was validated by immunohistochemistry, ELISA, and the injection of specific radiolabelled antibodies.

Results—Dexamethasone greatly reduced IL-1β induced leucocyte adhesion, endothelial expression of ICAM-1 in the postcapillary venule, and release of the mast cell derived chemokine KC. Injection of specific antibodies to the latter mediators was also extremely effective in downregulating (>80%) IL-1β induced leucocyte adhesion.

Conclusions—Induction by IL-1β of endogenous ICAM-1 and KC contributes to leucocyte adhesion to inflamed mesenteric vessels. Without excluding other possible mediators, these data clearly show that dexamethasone interferes with ICAM-1 expression and KC release from mast cells, resulting in suppression of leucocyte accumulation in the bowel wall, which is a prominent feature of several gastrointestinal pathologies.

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Keywords: inflammation; glucocorticoids; intravital microscopy; mast cell; neutrophil; endothelium

Glucocorticoid hormones are potent therapeutic tools in the management of inflammatory pathologies of the gastrointestinal tract, including inflammatory bowel disease. These and related pathologies are characterised by the presence of a prominent leucocyte infiltrate in the bowel wall. As glucocorticoid hormones show a potent ability to inhibit and suppress leucocyte accumulation into inflamed tissues,^{1 2} an effect on this component of the host inflammatory response may underline their therapeutic efficacy.

The accumulation of leucocytes in injured tissues is the result of a series of concerted events regulated by adhesion molecules expressed both on the circulating leucocyte and the vascular endothelium, and by the presence of specific leucocyte activators (chemokines).^{3 4} Videomicroscopy has shown that leucocytes roll on an inflamed vascular endothelium; this leads to firm adhesion and may be followed by emigration through the endothelial wall into the tissue.⁵ The three events of rolling, adhesion, and emigration are linked both functionally and temporally.⁶ The initial rolling is mediated by selectins that are expressed on leucocytes and the endothelium of the postcapillary venule; leucocyte firm adhesion is often sustained by β₂ integrins exposed on the leucocyte plasma membrane and their counter ligands, which include members of the immunoglobulin supergene family such as intercellular adhesion molecule 1 (ICAM-1).^{3 7} Chemokines are an important class of mediators which can show selectivity for different leucocyte subsets. Expression of specific chemokines can explain the selective cell accumulation characteristic of distinct pathologies. In particular, the CXC chemokines are involved in the extravasation of neutrophils. The archetypal CXC chemokine is interleukin (IL) 8⁸; KC (also known as cytokine induced neutrophil chemoattractant) is the major rat counterpart of interleukin 8.⁹

Interleukin 1 is a multipotent cytokine able to initiate the cascade of events listed above and to lead to intense accumulation of blood borne cells into injured tissues, including sections of the gut.¹⁰ Indeed, IL-1 plays an important role in experimental models of inflammatory bowel disease^{11 12}; this has been substantiated by clinical studies.^{13 14}

Using videomicroscopy we have shown that if direct acting chemoattractants (such as platelet activating factor or formylated peptides) are superfused onto the microvascular tissues of the rat mesentery to promote the leucocyte-endothelium interaction, treatment of animals with dexamethasone (DEX) affects selectively the leucocyte emigration process (also referred to as diapedesis), but not the rolling or adhesion processes.^{15 16} This effect of DEX seems to be due to increased amounts of lipocortin 1 in the adherent leucocyte,¹⁷ with consequent inhibition of the extent of cell transendothelial passage.^{15 18} When indirect leucocyte chemoattractants are used, such as

Abbreviations used in this paper: BSA, bovine serum albumin; DEX, dexamethasone; ICAM, intercellular adhesion molecule; IL, interleukin; PBS, phosphate buffered saline.

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IL-1 β , DEX is then also able to inhibit leucocyte adhesion to rat mesenteric postcapillary venules. However, doses of DEX in excess of 100 $\mu\text{g}/\text{kg}$ are required to observe this action which maximally reaches 50% inhibition.¹⁶

In the present study we sought to identify some of the molecular determinants responsible for the antiadhesive effect of DEX seen in mesenteric postcapillary venules exposed to IL-1 β . We hypothesised that potential targets for DEX action could be the adhesion molecule ICAM-1 and the CXC chemokine KC; the synthesis of both these mediators is induced by IL-1 β and is inhibited by DEX *in vitro*.^{19, 20}

Methods

ANIMALS

Male Sprague-Dawley rats (175–200 g body weight) were purchased from Interfauna (Huntingdon, UK) or from Charles River (Saint Aubin les Elbeuf, France) and maintained on a standard chow pellet diet with tap water *ad libitum*. All animals were housed for one week on a 12 hour light-dark cycle for acclimatisation prior to experimentation. For intravital microscopy studies all animals were starved for 24 hours prior to experimentation.

INTRAVITAL MICROSCOPY

The rat mesenteric preparation was set up as described recently.¹⁶ Rats were injected intraperitoneally with 20 ng rat recombinant IL-1 β (generous gift of Dr RC Newton, Du-Pont Merck, Wilmington, Delaware, USA) and were left at liberty prior to experimentation. Control animals received sterile saline alone (1 ml per rat intraperitoneally). Particular care was taken to observe the mesenteric vascular bed at exactly two or four hours after injection of the cytokine. Animals were anaesthetised with Inactin (sodium thiopentobarbital 120 mg/kg body weight intraperitoneally; RBI Natick, Massachusetts, USA); the neck and abdominal areas were gently shaved, and a tracheotomy was performed to facilitate breathing during experimentation. A midline abdominal incision was made and a loop of the ileal mesentery was exteriorised and superfused at a rate of 2 ml/min with warmed (37°C) bicarbonated buffer (pH 7.4), gassed with 5% CO₂/95% N₂. The exposed tissue was kept moist by covering with saline soaked gauze to minimise heat loss and fluid evaporation.

The animals were placed in a supine position on a Plexiglas viewing stage in preparation for microscopic observation; the mesentery was mounted onto a Zeiss Axioskop FS (Zeiss, Welwyn Garden City, UK) microscope stage. A water immersion objective lens (magnification $\times 40$; Zeiss) and an eyepiece (magnification $\times 40$; Zeiss) were used to observe the microcirculation. The preparation was transilluminated with a 12 V, 100 W halogen light source. A Hitachi CCD colour camera (model KPC571 Tokyo, Japan) acquired images that were displayed onto a Sony Triniton colour video monitor (model PVM 144OQM) and recorded onto a Sony super-VHS video cassette recorder (model SVO-9500 MDP) for subsequent offline analysis. A video time-date generator

(FOR-A video timer, model VTG-33, Tokyo, Japan) projected the time, date, and stopwatch functions onto the monitor. Three to five randomly selected postcapillary venules (diameter 20–40 μm ; length at least 100 μm) were observed for each rat. The extent of the inflammatory response elicited by IL-1 β prevented evaluation of the rolling phenomenon with quantification of white blood cell velocity. In contrast, adhesion could be easily monitored by counting, for each vessel, the number of adherent leucocytes in 100 μm length. Leucocyte emigration from the microcirculation into the tissue was quantified by counting the number of cells that had emigrated out of the vessel at up to 50 μm , 50–100 μm , and 100–150 μm away from the vessel wall in parallel with 100 μm vessel segments.

In some experiments the number of peritoneal leucocytes was also monitored by staining aliquots of the peritoneal lavage fluids (collected by washing the cavities with 10 ml phosphate buffered saline (PBS)) with Turk's solution (0.01% crystal violet in 3% acetic acid) and counting cells under a light microscope.

DRUG TREATMENTS

Dexamethasone (sodium phosphate salt, David Bull Laboratories, Warwick, UK) was administered subcutaneously at 30 or 100 $\mu\text{g}/\text{kg}$ one hour prior to IL-1 β .¹⁶ The functional role of endogenous ICAM-1 was assessed by injecting 2 mg/kg intravenously of mouse anti-rat ICAM-1 monoclonal antibody (clone 1A29) 30 minutes prior to challenge with 20 ng rat IL-1 β intraperitoneally; both dose and protocol of treatment have been validated in several previous studies—for example, Zimmerman *et al.*²¹ The potential functional role for endogenous KC was determined by injecting 2 mg intravenously of specific goat anti-rat KC polyclonal antibody (a generous gift of Dr John Zagorski, Oral Infection and Immunity Branch, NIDR/NIH, Bethesda, Maryland, USA) 30 minutes prior to challenge with the cytokine.²² Control animals received identical doses of non-immune mouse IgG or goat IgG (Sigma Chemical Co., Poole, UK), respectively.

WHOLE MOUNT PREPARATION OF MESENTERIES TO DETECT ICAM-1 AND KC EXPRESSION

ICAM-1

As detection of endothelial antigens has been successfully obtained following *in vivo* administration of the primary antibody (for example, P-selectin²³), rats were treated with an anti-ICAM-1 monoclonal antibody (clone 1A29; 2 mg/kg intravenously) 10 minutes prior to tissue collection. Animals were perfused through the heart with 100 ml of 4% paraformaldehyde in 0.1 M PBS at room temperature. The mesentery was then ligated and removed; the appropriate ileal section of the tissue was carefully excised and pinned out onto Sylgard 184 elastomer resin (BDH, Poole, UK) and postfixed in the same fixative. Tissues were permeabilised in graded ethanol and endogenous peroxidases quenched with 1% H₂O₂ for 30 minutes. After thorough washing, whole

Table 1 Effect of dexamethasone (DEX) on interleukin (IL) 1 β induced leucocyte extravasation into the rat peritoneal cavity

Treatment	Time (h)	Leucocytes	
		10 ⁶ per rat	%
None	0	0.97 (0.06)	100
IL-1 β	2	1.48 (0.27)	152
+ DEX	2	1.22 (0.17)	126
IL-1 β	4	3.10 (0.25)*	319
+ DEX	4	1.10 (0.29)†	113

Rats were left untreated or injected intraperitoneally with 20 ng rat IL-1 β . In some cases animals were pretreated with DEX (100 μ g/kg subcutaneously) one hour prior to cytokine administration. Data are mean (SEM); n=6 rats per group.

*p<0.05 versus control group (time 0); †p<0.05 versus respective IL-1 β group (time 4 h).

mounts were incubated with normal horse serum (1/100 in PBS + 0.1% low endotoxin bovine serum albumin, globulin free) for one hour to block non-specific binding sites for IgGs. Mounts were then drained and incubated with secondary preadsorbed biotinylated horse antimouse IgG (Vectastain Elite ABC, Vector Laboratories, Peterborough, UK) at 4°C overnight. Following another washing step in PBS, tissues were first incubated with ABC horse-radish peroxidase complex (30 minutes) and then with 0.05% 3,3'-diaminobenzidine tetra-

hydrochloride (Sigma Chemical Co.) in 0.05 M Tris buffer for colour development.

KC

In order to monitor KC expression, whole mount preparations were prepared as above, peroxidase blocked, and incubated in vitro with normal goat serum (1/66.7 in PBS + 0.1% low endotoxin bovine serum albumin, globulin free) to block non-specific binding sites for IgGs. Tissues were then incubated overnight at 4°C with a rabbit antirat KC polyclonal antibody (0.5 μ g/ml; gift of Dr K Watanabe, Institute for Cytosignal Research, Tokyo, Japan). The remainder of the experiment was as above, with the exception that a goat antirabbit IgG was used as secondary antibody (Vectastain Elite ABC, Vector Laboratories, Peterborough, UK).

In all cases, preparations were treated according to the strict protocol outlined above to reduce possible variabilities between the whole mounts. All tissues were counterstained with haematoxylin and mounted with an aqueous mounting medium.

MEASUREMENT OF ENDOTHELIAL ICAM-1

EXPRESSION BY IN VIVO DUAL RADIOLABELLING

Mouse antirat ICAM-1 monoclonal antibody (clone 1A29) and clone P-23 (a non-binding murine IgG directed against human P-selectin, used as internal control) were labelled with ¹²⁵I and ¹³¹I, respectively by an established method.^{24 25} Rats were injected intraperitoneally with 20 ng IL-1 β , and two or four hours later were anaesthetised with 100 mg/kg intraperitoneal Inactin, and the right carotid artery and jugular vein were cannulated. A mixture of 4 μ g ¹²⁵I-ICAM-1 monoclonal antibody, 5 μ g ¹³¹I-non-binding monoclonal antibody (P-23), and 246 μ g unlabelled ICAM-1 monoclonal antibody was administered via the jugular vein catheter. After five minutes, blood samples were obtained through the carotid artery. Animals were exsanguinated by vascular perfusion with sodium bicarbonate buffer via the jugular vein with simultaneous blood withdrawal via the carotid artery. The inferior vena cava was severed and the animal constantly perfused with sodium bicarbonate buffer via the carotid artery until all blood was flushed. To calculate ICAM-1 expression, ¹²⁵I (binding antibody) and ¹³¹I (non-binding antibody) activities in each organ or tissue and in a 50 μ l plasma sample were estimated using a Cobra II gamma counter (Packard, Meriden, Australia), with automatic correction for background activity and spillover. The total radioactivity injected in each experiment was also calculated by counting a 5 μ l sample of the mixture containing the radiolabelled monoclonal antibodies. The accumulated activity of each monoclonal antibody in a select organ was expressed as the percentage of the injected dose (% ID) per g of wet tissue. The formula used to calculate ICAM-1 expression was as follows: ICAM-1 expression = (% ID/g for ¹²⁵I) - (% ID/g for ¹³¹I) \times (% ID ¹²⁵I in plasma)/(% ID in ¹³¹I plasma).

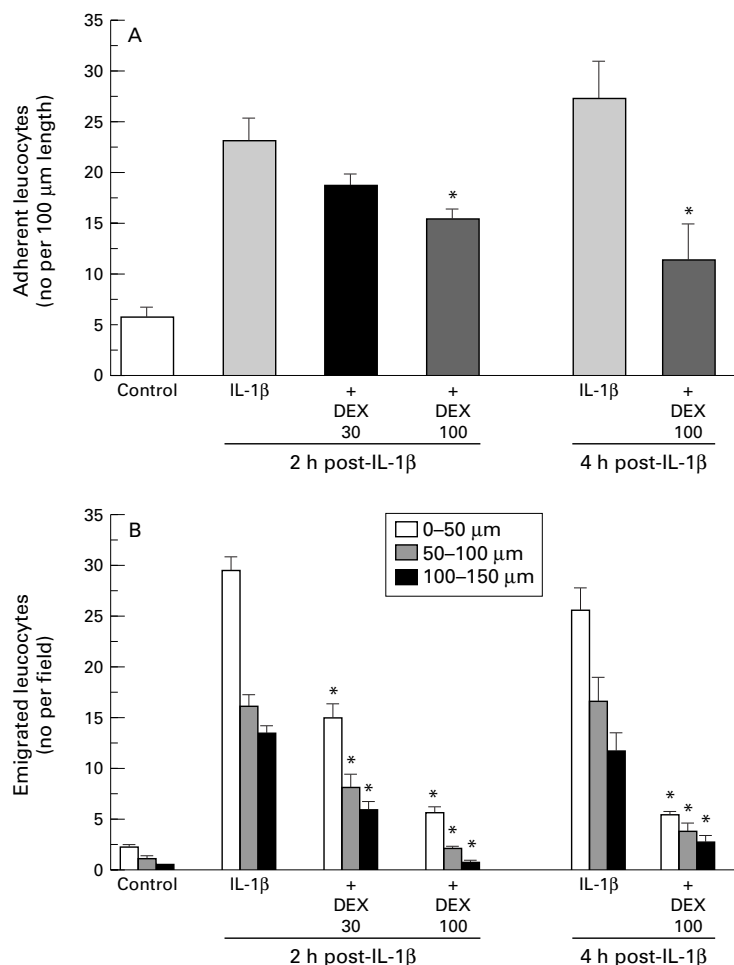


Figure 1 Effect of dexamethasone (DEX) on interleukin (IL) 1 β induced leucocyte adhesion and emigration in rat mesenteric postcapillary venules. Data are mean (SEM); n=6 rats. *p<0.05 v respective IL-1 β group.

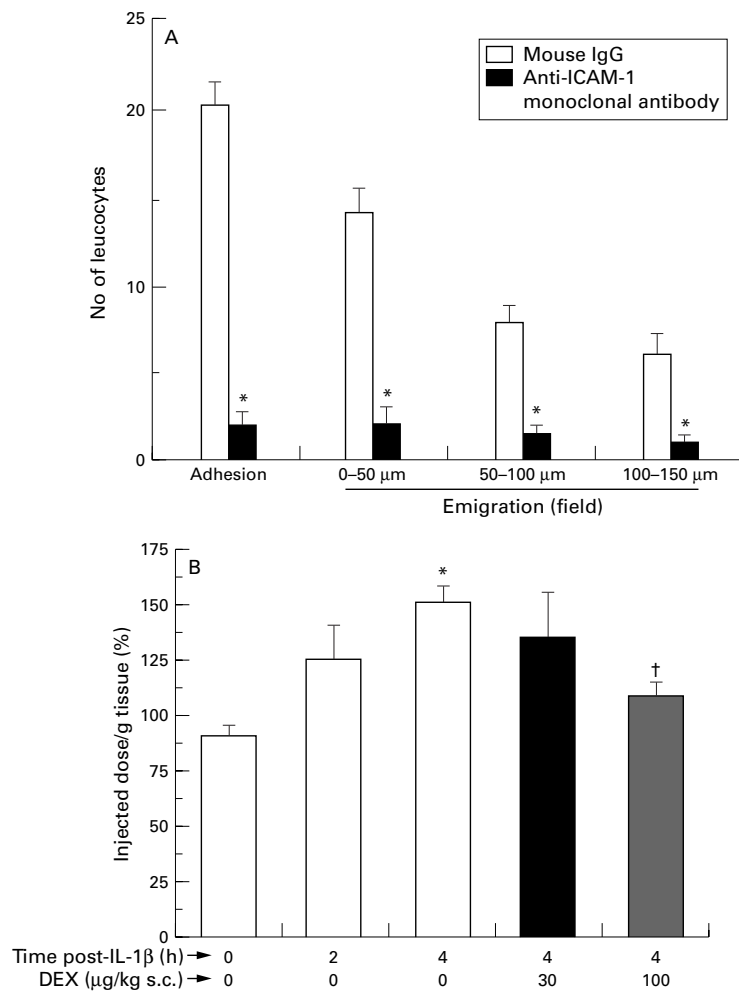


Figure 2 Involvement of intercellular adhesion molecule (ICAM) 1 in interleukin (IL) 1 β induced cell adhesion and emigration. (A) Animals were treated with 2 mg/kg mouse IgG or mouse antirat ICAM-1 monoclonal antibody one hour prior to IL-1 β administration (20 ng intraperitoneally). Mesenteries were exposed two hours later, and the degree of cell adhesion (per 100 μ m vessel wall) and emigration quantified by videomicroscopy. Data are mean (SEM); n=6 rats. *p<0.05 v mouse IgG group. (B) ICAM-1 expression in the mesenteric tissue as assessed by the dual antibody technique. Rats were treated with rat IL-1 β (20 ng intraperitoneally) and some of them pretreated with dexamethasone (DEX) one hour prior to the cytokine. At the reported times post-IL-1 β , the specific radioactivity due to endogenous ICAM-1 was determined. Data are mean (SEM); n=5 rats per group. *p<0.05 v control group (time 0); †p<0.05 v IL-1 β group (time 4 h).

ELISA FOR KC DETECTION

The amounts of the KC chemokine in cell free peritoneal lavage fluids were quantified using a murine KC ELISA (R&D, Oxon, UK). A standard curve with rat synthetic KC (gift of Dr K Watanabe, Institute for Cytosignal Research, Tokyo, Japan)²⁶ was constructed (concentration range 1–1000 pg/ml) and compared with that of murine KC (furnished by the manufacturer): 100% cross reactivity with rat KC was found. Data are reported as pg of rat KC per cavity.

STATISTICS

Data are reported as mean (SEM) of the number of rats per group. Statistical differences were assessed by one way analysis of variance followed by the Bonferroni test for intergroup comparisons. A value of p<0.05 was taken as significant.

Results

EFFECT OF DEXAMETHASONE

Intraperitoneal injection of IL-1 β caused a generalised inflammatory response with an increase in the number of adherent cells at two hours (fourfold) and four hours (fivefold) above that of saline treated animals (fig 1A). Pretreatment with DEX one hour prior to cytokine administration caused a dose dependent decrease in the number of adherent cells at the two hour time point which reached significance only at the 100 μ g/kg dose. The inhibitory effect of DEX on IL-1 β induced cell adhesion was slightly greater at the four hour time point (approx. 60%; fig 1A). IL-1 β caused an increase in the number of emigrated cells outside the postcapillary venule at two and four hours. As expected, the number of emigrated cells was lower in the zones further away from the postcapillary wall (fig 1B). In

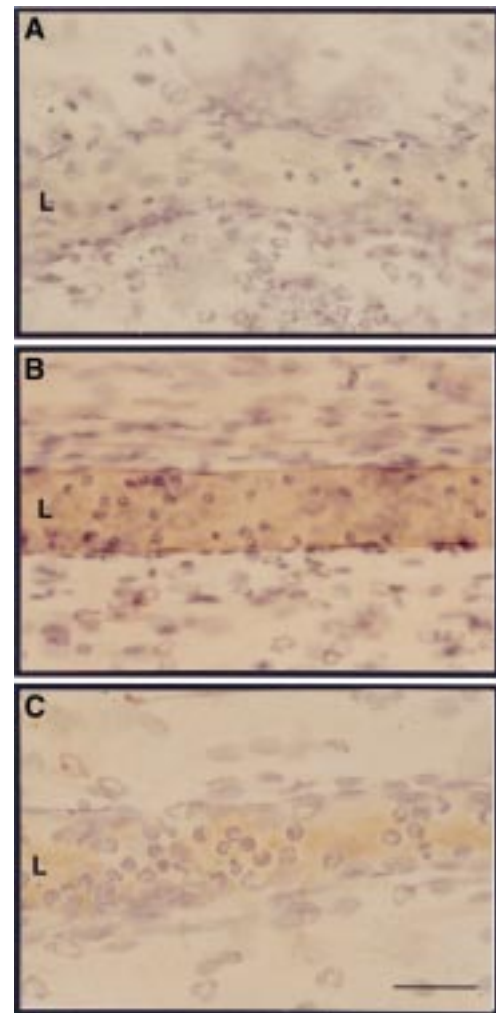


Figure 3 Localisation of intercellular adhesion molecule (ICAM) 1 immunostaining in inflamed rat mesenteric postcapillary venules. Typical micrographs of the ileal section of rat mesentery whole mounts. (A) Preparations excised two hours post-administration of 20 ng rat interleukin (IL) 1 β from an animal injected with control mouse IgG 10 minutes prior to sacrifice. (B) As for A, but the rat was injected with 2 mg/kg mouse antirat ICAM-1 10 minutes prior to sacrifice and tissue collection. Note the brown staining around the endothelium of the postcapillary venule. (C) As in B, but the rat was treated with 100 μ g/kg subcutaneous dexamethasone one hour prior to intraperitoneal injection of IL-1 β . Note the scarce brown immunostaining compared with B. Pictures are representative of five distinct preparations. L, vessel lumen. Bar, 30 μ m.

Table 2 Effect of dexamethasone (DEX) and intercellular adhesion molecule (ICAM) 1 expression in several abdominal organs after administration of interleukin (IL) 1 β

Treatment (h post IL-1 β)	Liver	Pancreas	Stomach	Jejunum	Ileum	Caecum	Distal colon
Control (0)	2.99 (0.15)	0.09 (0.01)	0.09 (0.01)	0.15 (0.02)	0.14 (0.02)	0.12 (0.01)	0.09 (0.01)
IL-1 β (2)	3.83 (0.27)	0.13 (0.01)	0.10 (0.01)	0.19 (0.01)	0.19 (0.01)	0.20 (0.02)	0.12 (0.01)
IL-1 β (4)	4.28 (0.41)*	0.17 (0.02)*	0.11 (0.01)	0.22 (0.03)*	0.22 (0.03)*	0.16 (0.01)	0.12 (0.01)
+ 30 μ g/kg DEX (4)	3.23 (0.21)†	0.20 (0.02)	0.12 (0.02)	0.29 (0.04)	0.22 (0.40)	0.18 (0.02)	0.13 (0.02)
+ 100 μ g/kg DEX (4)	3.30 (0.20)†	0.13 (0.01)	0.09 (0.01)	0.19 (0.03)	0.15 (0.01)†	0.16 (0.01)	0.10 (0.01)

Rats (n=5) were left untreated (control group) or were injected intraperitoneally with 20 ng rat IL-1 β at time 0. Other animals received DEX at the reported doses subcutaneously one hour prior to IL-1 β . Data (mean (SEM)) are reported as percentage of injected dose/g wet tissue.

*p<0.05 versus control group (time 0); †p<0.05 versus IL-1 β group (4 h).

contrast to cell adhesion, the lower dose of 30 μ g/kg DEX inhibited cell emigration at two hours (50%; fig 1B). Similarly, the higher dose of 100 μ g/kg caused a greater inhibition in the number of emigrated cells (approximately 80% reduction). With either dose of DEX the number of emigrated cells was significantly reduced in all fields measured. No difference in the number of cells collected from the peritoneal cavity was seen at two hours post-IL-1 β whereas a significant increase was measured in the IL-1 β group at the four hour time point (table 1). This effect was also abolished by treatment with 100 μ g/kg DEX.

ROLE OF ENDOGENOUS ICAM-1 AND THE EFFECT OF DEXAMETHASONE

Passive immunisation of rats against ICAM-1 provoked a remarkable inhibition of IL-1 β induced leucocyte adhesion (90% reduction) and emigration (>80% reduction), as assessed at the two hour time point (n=6, p<0.05; fig 2A). A notable immunostaining for ICAM-1 was associated with the endothelium in whole mounts of postcapillary venules inflamed with IL-1 β (fig 3B); this was not seen in control tissues (where rats were treated intravenously with mouse IgG) (fig 3A). Treatment of rats with DEX (100 μ g/kg subcutaneously, one hour) reduced the intensity of the immunostaining associated with the inflamed vessels (fig 3C).

To quantify the latter observation, the dual antibody technique was used. Figure 2B shows the increased radioactivity associated with the mesenteries at two and four hours post-IL-1 β injection. As statistical significance was reached at four hours post-IL-1 β , the effect of DEX was tested only at this time point. Treatment of rats with DEX produced a significant reduction in IL-1 β induced ICAM-1 expression only at the highest dose tested, that is, 100 μ g/kg (>70% inhibition if calculated on the net values, when the basal level was subtracted; n=4, p<0.05; fig 2B). The glucocorticoid hormone did not affect basal (constitutive) expression of ICAM-1 when injected in control rats (data not shown). Table 2 reports the dual effect of IL-1 β and DEX on ICAM-1 expression in several organs of the peritoneal cavity. High constitutive ICAM-1 was found in the liver, with lowest values being measured in stomach, pancreas, and distal colon. Intraperitoneal injection of IL-1 β induced ICAM-1 expression in a time dependent fashion in all organs tested except stomach, caecum, and distal colon. The highest increment was seen in

the pancreas (+88%), followed by the liver (+45%), ileum (+57%), and jejunum (+47%) (table 2). Treatment of rats with the highest dose of DEX prevented IL-1 β induced ICAM-1 induction in most organs except the jejunum. At the lower dose of 30 μ g/kg, DEX was found to be inhibitory only in the liver.

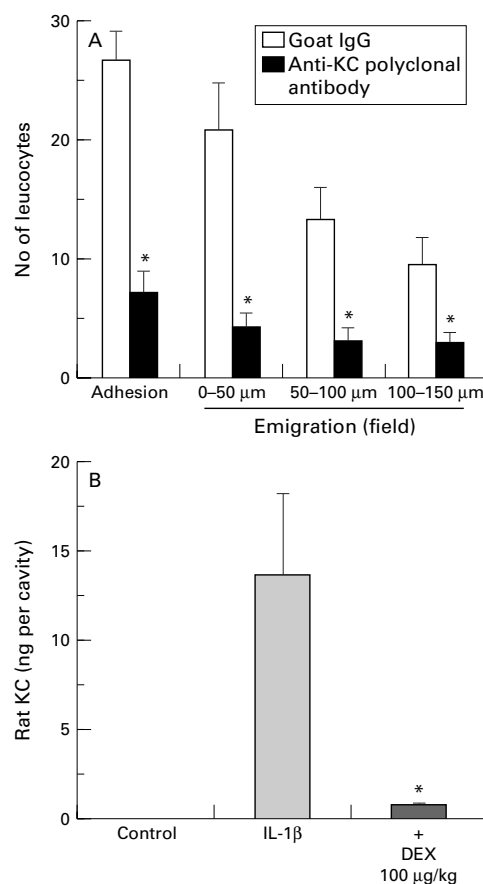


Figure 4 Involvement of KC in interleukin (IL) 1 β induced cell adhesion and emigration. (A) Animals were treated with 2 mg goat IgG or goat antirat rat KC polyclonal antibody one hour prior to IL-1 β administration (20 ng intraperitoneally). Mesenteries were exposed two hours later, and the degree of cell adhesion (per 100 μ m vessel wall) and emigration quantified by videomicroscopy. Data are mean (SEM); n=8 rats. *p<0.05 v mouse IgG group. (B) Detection of rat KC protein in the peritoneal cell free lavage fluids of control rats (n=4), rats injected with IL-1 β (20 ng intraperitoneally; n=5), and animals pretreated with dexamethasone (DEX) one hour prior to IL-1 β administration (n=5). Lavage fluids were collected two hours post-IL-1 β . Data are mean (SEM). *p<0.05 v IL-1 β group.

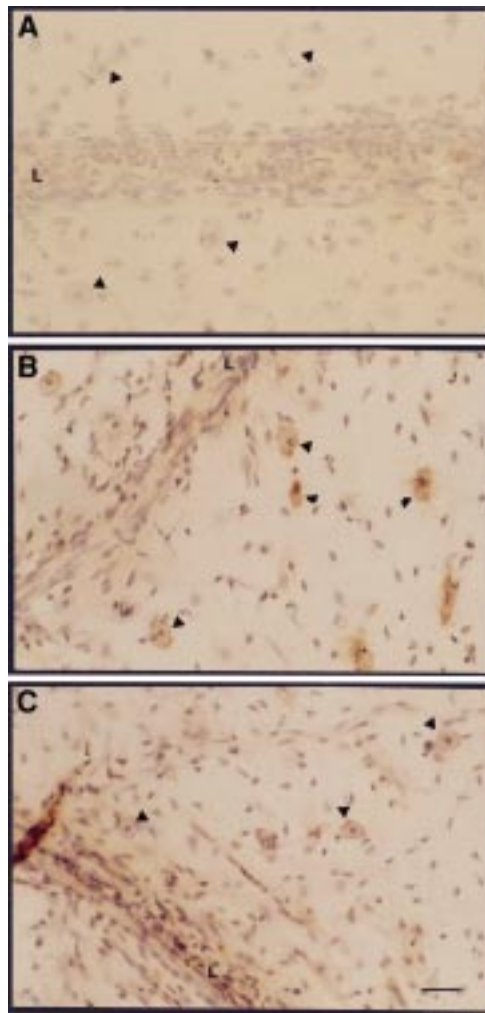


Figure 5 Localisation of KC immunostaining in inflamed rat mesenteric postcapillary venules. Typical micrographs of the ileal section of rat mesentery whole mounts. (A) Preparations excised two hours after administration of 20 ng rat interleukin (IL) 1 β and incubated with non-specific rabbit IgG. Arrowheads indicate some of the perivenular mast cells. (B) As in A, but the tissue was incubated with specific rabbit antirat KC IgG. Arrowheads indicate some of the perivenular mast cells which stained for KC. (C) As in B, but the rat was treated with 100 μ g/kg subcutaneous dexamethasone one hour prior to intraperitoneal injection of IL-1 β . Arrowheads indicate some of the perivenular mast cells which appeared less stained for KC when compared with B. Pictures are representative of four distinct preparations. L, vessel lumen. Bar, 25 μ m.

ROLE OF ENDOGENOUS KC AND THE EFFECT OF DEXAMETHASONE

The *in vivo* administration of anti-KC polyclonal antibody produced a fourfold reduction in IL-1 β induced cell adhesion and emigration in the rat mesenteries at two hours as quantified by intravital microscopy (n=8, p<0.05; fig 4A). To visualise the presence of endogenous KC after IL-1 β administration, mesenteries were immunostained with a specific anti-KC rabbit serum. Figure 5B shows the intense immunostaining found in perivenular mast cells which was absent in the inflamed tissue stained with normal goat serum (fig 5A). In some cases KC positivity seems to be associated with the granules which have been released from the activated mast cell (fig 5B). Treatment of rats with DEX (100 μ g/kg subcutaneously) reduced KC immunostaining (fig 5C). To substantiate

these observations in a quantitative manner, the release of KC protein in the peritoneal lavage fluids was also monitored. Figure 4B shows that KC immunoreactivity was not detected in saline injected rats (control), whereas a notable release was measured two hours post-IL-1 β injection. This was almost completely abrogated by 100 μ g/kg DEX (n=5, p<0.05; fig 4B).

Discussion

In this study we showed for the first time that endothelial ICAM-1 and mast cell derived KC mediate IL-1 β induced cell adhesion to inflamed postcapillary venules in the gut. We also showed that the inhibitory effect of DEX on these parameters is functionally linked to a reduction of leucocyte adhesion to these vessels.

Intraperitoneal injection of IL-1 β produced an intense local inflammatory response which was essentially maximal at the two hour time point when monitored by videomicroscopy. This technique has been previously used to characterise the molecular determinants involved in this response. A functional role for endothelial platelet-endothelial cell adhesion molecule 1 (CD31) and platelet activating factor in the IL-1 β induced cell emigration, but not cell adhesion, process induced by IL-1 β has been reported previously.^{10, 27} No studies have addressed the nature of the mediators responsible for IL-1 β induced leucocyte adhesion. Among several potential candidates, we focused on ICAM-1 (CD54) for the following reasons: it is induced *in vitro* by IL-1 β ²⁸; it is functionally related to IL-1 β induced neutrophil transendothelial migration *in vitro*^{29, 30}; and a neutralising monoclonal antibody antirat ICAM-1 well characterised in *in vivo* systems was available.²¹ We found that IL-1 β induced leucocyte adhesion *in vivo* is largely dependent on ICAM-1. As expected, an intense expression of this adhesion molecule on the postcapillary endothelium of rat mesenteries exposed to IL-1 β was seen by immunohistochemical analysis.

Among other mediators, glucocorticoid hormones also target adhesion molecules to inhibit the interaction between leucocytes and endothelium. Until now, this has been mainly shown *in vitro*; the pioneering study of Cronstein *et al*³¹ reported DEX inhibition of ICAM-1 and E selectin (CD62E) expression on activated endothelial cells. This effect of DEX has been functionally related to cell adhesion³¹ and emigration³² as assessed under flow conditions *in vitro*. Few studies have investigated the effects of DEX on adhesion molecule expression *in vivo*. Suzuki *et al*³³ showed endogenous glucocorticoid hormones to control P selectin expression in rat mesenteric postcapillary venules. Here, we have initially confirmed the dose dependency for DEX inhibition of IL-1 β induced cell adhesion such that a dose of 100 μ g/kg was required to reduce this parameter (whereas a significant inhibition of IL-1 β induced cell emigration was seen at a dose of 30 μ g/kg).¹⁶ Treatment of rats with 100 μ g/kg DEX seemed to reduce the extent of ICAM-1 immunostaining observed

on the activated postcapillary venule. This observation was substantiated in a quantitative manner by the experiments with radiolabelled antibodies. The dual antibody radiolabelling technique has been recently refined and used to measure endothelial ICAM-1 expression in large vascular beds and organs following animal treatment with lipopolysaccharide or tumour necrosis factor α .²⁴⁻³³ In our case a basal expression of ICAM-1 was detected in inactivated rat mesenteries, and values were increased following IL-1 β injection to reach statistical significance at four hours postinjection. The effect of DEX was then assessed at this time point as an almost identical inhibition of IL-1 β induced cell adhesion was seen by videomicroscopy at two and four hours post-IL-1 β . In keeping with the functional data, a significant attenuation of anti-ICAM-1 monoclonal antibody binding associated with the rat mesenteries was measured only after administration of the antiadhesive dose of DEX (100 μ g/kg). Overall these data indicate that: IL-1 β induces ICAM-1 on the postcapillary endothelium of rat mesenteries and this is functionally linked to leucocyte adhesion (and emigration in view of the sequential relation between these events); and treatment of rats with 100 μ g/kg DEX can prevent induction of this adhesion molecule which is correlated with its inhibitory effect on cell adhesion.

The CXC chemokine KC is a potent neutrophil activator in vitro,³⁴⁻³⁵ and recruits leucocytes on the endothelium of postcapillary venules once superfused on rat mesenteries.³⁶ The link between KC and IL-1 β , however, has been mainly investigated in in vitro studies, reporting the clear ability of the cytokine to induce KC expression and release.²⁰⁻³⁷ We now show that endogenous KC mediates IL-1 β induced cell adhesion to the activated mesenteric endothelium in vivo using a polyclonal antibody validated in a rat model of peritonitis.²² A similar degree of inhibition (70–90%) of IL-1 β induced leucocyte adhesion and emigration was obtained with the anti-ICAM-1 monoclonal antibody or with the anti-KC polyclonal antibody; this indicates that there is a clear redundancy of mediators which are activated by the cytokine to promote the sticking of rolling leucocytes, or that the expression and/or function of these two mediators is somehow linked. There are no reports that KC can induce ICAM-1 protein expression, but as the chemokine can upregulate ICAM-1 ligand (the β_2 integrin complex exposed on the leucocyte cell surface)³⁸ the latter hypothesis seems plausible.

The immunohistochemical analysis showed KC expression to be associated with perivascular mast cells, tissue dwelling leucocytes which play a central role in initiation of the host inflammatory response.³⁹ The identity of this cell type was also confirmed by the classical metachromatic staining of the granules with toluidine blue (not shown). To our knowledge this is the first study which shows KC (or any other chemokine) association with mast cells in experimental inflammation in vivo. Until now, few in vitro studies have shown the ability of mast cells to produce chemokines, although

there is evidence that a human mast cell leukaemia line can express multiple chemokine genes⁴⁰ and that interleukin 8 is then stored in granules and released on activation.⁴¹

Treatment of rats with DEX seemed to reduce the extent of KC immunostaining observed in perivascular mast cells. These qualitative observations were reinforced by the ELISA experiments. Rat KC protein was not detected in the lavage fluids of control rats, but it increased two hours after local administration of IL-1 β . This effect was essentially abrogated by DEX. It has been previously reported that DEX is able to prevent rat KC gene expression²⁰ through an impairment of nuclear factor KB activation.⁴² In addition, glucocorticoid hormone inhibition of this nuclear factor is also at the basis of the negative effect on ICAM-1 upregulation in in vitro experiments.⁴³ It is very likely that similar molecular mechanisms are operating in our experimental conditions in vivo. If this hypothesis is confirmed in future studies, we would then know that a dose of 100 μ g/kg DEX has to be used experimentally to affect nuclear factor KB mediated events in vivo. These observations add KC to the list of chemokines, including macrophage inflammatory protein 1 α or monocyte chemoattractant protein 1⁴⁴ affected by in vivo treatment with DEX, a feature, however, which is not shared by all chemokines.⁴⁵

To conclude, glucocorticoid hormones are potent anti-inflammatory drugs which are widely used in several pathologies of the bowel.⁴⁶ Here we have used an experimental model relevant to these pathologies in view of the central role played by IL-1 β ¹¹⁻¹³ and confirmed an effect of DEX on the leucocyte extravasation process activated by this cytokine. We cannot exclude that other adhesion molecules and/or chemokines might be involved in the antiadhesive effect of DEX, although with these doses and protocol of treatment we can certainly eliminate an effect of the glucocorticoid hormones on expression of leucocyte adhesion molecules.⁴⁵⁻⁴⁷ Using a series of in vitro and in vivo assays, we show that inhibition of ICAM-1 and KC expression and/or release is at least one of the basic mechanisms responsible for a major therapeutic action of glucocorticoid hormones, which is the inhibition of leucocyte infiltration into injured tissues.

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