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Dominant role of L- and P-selectin in mediating CXC chemokine-induced neutrophil migration *in vivo*

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1 The role of selectins in neutrophil emigration in response to the CXC chemokines KC and MIP-2 was investigated in wild type and P-selectin deficient mice.

2 Intrapleural injection of KC or MIP-2 induced a rapid and specific neutrophil accumulation. Emigration 2 h after KC or MIP-2 was reduced 83–88% by anti-L-selectin mAb and 53–63% by anti-P-selectin mAb. Co-administration of anti-L- and P-selectin mAbs abolished neutrophil migration induced by either chemokine.

3 An anti-E-selectin mAb tested alone did not affect KC-induced neutrophil migration after 2 or 4 h. Moreover, anti-E-selectin did not have an additive inhibitory effect on KC-induced neutrophil migration compared with P-selectin blockade alone. This was found when neutrophil migration was measured at 2 and 4 h after KC.

4 Despite a blood neutrophilia, neutrophil migration at 2 and 4 h after KC was markedly smaller (by approximately 90%) in P-selectin deficient mice compared with wild type animals. Responses at both time points were not decreased further in animals given E-selectin mAb but were reduced to the PBS control level in the presence of anti-L-selectin.

5 *In vitro* study of cultured murine endothelial cells demonstrated that KC can directly increase cell surface P-selectin expression.

6 These data suggest that CXC chemokine-induced neutrophil accumulation is dependent on both neutrophil L-selectin and a rapid upregulation of endothelial P-selectin but there is no evidence for E-selectin induction.

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Abbreviations: KC, product of KC gene; mAb, monoclonal antibody; MIP-2, macrophage inflammatory protein-2; PBS, phosphate buffered saline

Introduction

The accumulation of leukocytes, followed by their migration out of the vasculature into surrounding tissue, where they can effect damage, is a characteristic feature of the inflammatory response. Chemoattractants, including chemokines, play a key role in the complex network of events leading to leukocyte adhesion and emigration. The chemokines can be divided into four families: the CXC or α family is primarily chemotactic for neutrophils, whereas members of the CC or β family predominately act on monocytes, macrophages, T-lymphocytes and eosinophils (Bacon & Schall, 1996; Baggiolini, 1998; Luster, 1998; Rollins, 1997). C and CX_3C chemokines also exist although their function is less well characterized.

CXC chemokines have been implicated in many inflammatory diseases where the neutrophil is a propagator of tissue damage (Feng *et al*., 1995; Gupta *et al*., 1996; Lukacs *et al*., 1998; Luster, 1998; Rollins, 1997; Tessier *et al*., 1997). The most extensively studied member of this family is interleukin 8 (IL-8). The pro-inflammatory actions of human IL-8 are mediated through the binding to selective receptors of which two have been cloned; CXCR1, that binds IL-8 and GCP-2 with high affinity (Holmes *et al*., 1991; Murphy, 1996; Wuyts *et al*., 1998) and CXCR2 that binds many of the CXC chemokines (IL-8, GROα, GROβ, GROγ, NAP-2, ENA78, GCP-2) with similar affinities (Lee *et al*., 1992; Wuyts *et al*., 1998). Both receptor types are expressed in abundance on human neutrophils (reviewed in Murphy, 1996) and the CXC chemokines listed above are potent activators of neutrophil chemotaxis, shape change, respiratory burst and granule enzyme release (Baggiolini, 1998; Bozic *et al*., 1995). *In vitro* investigations have also demonstrated that IL-8 can promote L-selectin shedding with simultaneous upregulation of β_2 integrins on human neutrophils (Jutila *et al*., 1990; Kishimoto *et al*., 1989); these processes are considered to be important in the process of neutrophil accumulation at sites of inflammation.

Human IL-8 has also been shown to induce L-selectin shedding from activated mouse neutrophils *in vitro* (Watson *et al*., 1991). However, no CXCR1 receptor or IL-8 homologue has been identified in the mouse. A murine CXCR2 receptor homologue was reported by Ceretti *et al*. (1993) and subsequently shown to be the receptor on murine neutrophils capable of binding the murine CXC

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chemokines KC and MIP-2 with high affinity (Bozic *et al*., 1994; Lee *et al*., 1995). KC and MIP-2 are considered to be the murine equivalents of human GROα and GROβ/γ, respectively.

Previously, we demonstrated that LPS-induced neutrophil accumulation in the mouse pleural cavity was dependent on functional expression of the selectins, since blockade of L-selectin or combined blockade of P- and E-selectin inhibited the influx of neutrophils at 4 h (Henriques *et al*., 1996). Endothelial selectins are not constitutively expressed, with the exception of E-selectin in the cutaneous microcirculation (Norton *et al*., 1991). Thus, the capacity of LPS to upregulate P- and E-selectin expression *in vivo* (Gotsch *et al*., 1994; Labow *et al*., 1994; Mayadas *et al*., 1993; Sanders *et al*., 1992) could explain the inhibitory effect of monoclonal antibodies (mAbs) against these molecules on LPS-induced neutrophil recruitment. It is not known, however, whether selectins are similarly required for neutrophil accumulation induced by a direct-acting inflammatory mediator such as a CXC chemokine. There is evidence that chemokines may modulate the expression of cell adhesion molecules, since neutralization of endogenous MIP-1α in LPS-treated mice results in a decrease in ICAM-1 mRNA levels in lung tissue (Standiford *et al*., 1995). However, direct regulation of selectin function *in vivo* by a chemoattractant has not been extensively studied.

The aim of the present investigation was to assess the contribution of selectins to neutrophil migration into the mouse pleural cavity induced by a CXC chemokine. We have studied the dose dependency and kinetics of accumulation in response to KC and MIP-2. In addition, we have examined the role of the selectins in mediating the recruitment by use of specific anti-murine selectin mAbs and mice deficient in P-selectin. We also investigated the capacity of KC to induce surface expression of P-selectin on murine endothelial cells *in vitro*.

Methods

Animals

BALB/c and C57BL/6J mice were obtained from Harlan (Bicester, U.K.). P-selectin deficient mice (P-selectin $-/-$) (C57BL/6J-*Selp*) (Bullard *et al*., 1995) were obtained from The Jackson Laboratory (Bar Harbor, ME, U.S.A.). Mice weighed 18–22 g when used for study.

Reagents

KC and MIP-2 were purchased from R&D systems (Abingdon, U.K.). Stock solutions of KC (100 μg ml^{-1}) were prepared in sterile phosphate buffered saline (PBS; Life Technologies, Paisley, U.K.) containing 0.1% low endotoxin BSA (Sigma) and aliquots were stored at -20°C until further use. The endotoxin concentration of these stock solutions was less than than 0.1 EU endotoxin ml^{-1}. Heparin was from Leo Laboratories (Princes Risborough, U.K.). RPMI, DMEM, FCS, non-essential amino acids, penicillin/streptomycin and β-mercaptoethanol were from Life Technologies.

mAbs

MEL-14 (Gallatin *et al*., 1983) hybridoma (ATCC, Rockville, MD, U.S.A.) was grown in a hollow fibre bioreactor and mAb was purified by ammonium sulphate precipitation. The final MEL-14 concentration was 1 mg ml^{-1} and was injected i.v. at a dose of 100 μg per mouse 30 min before further treatment (Henriques *et al*., 1996). Rat IgG (Sigma) was used as a control.

Rat IgG1 to murine P-selectin, both the blocker 5H1 and the non-blocker 10A10, were prepared as described (Labow *et al*., 1994); the stock concentrations were 17.8 and 6.9 mg ml^{-1}. For the endothelial cell P-selectin expression experiments, rat IgG1 to murine P-selectin (RB40.34), obtained from BD Pharmingen (Oxford, U.K.), was also used. Rat IgG2b to murine E-selectin (10E6, a blocker) was prepared as described (Norton *et al*., 1993); the stock concentration was 18.3 mg ml^{-1}. A non-blocking anti-murine E-selectin mAb (14E4, rat IgG2a) was also used; the stock concentration was 7.3 mg ml^{-1}. P- and E-selectin mAbs were administered i.v. at a dose of 30 μg per mouse 15 min before intrapleural injection of chemokines. We have shown these doses of blocking mAb to suppress neutrophil accumulation in the mouse without an effect on circulating cells (Henriques *et al*., 1996).

Alexa Fluor® 488 labelled goat anti-rat IgG was obtained from Molecular Probes (Eugene, OR, U.S.A.).

Pleural inflammation

Inflammation in the pleural cavity was induced by injection of either KC or MIP-2. An adapted needle was inserted carefully through the parietal pleura into the left side of the thoracic cavity for the injection of chemokines (in 50 μl sterile PBS). Control animals received an equal volume of sterile PBS only. At 2, 4 or 6 h, the animals were killed by an overdose of sodium pentobarbitone. The pleural cavity was then lavaged with 1 ml r.p.m.i. containing heparin (10 iu ml^{-1}). Total leukocyte counts were determined using a haemocytometer and differential counts on stained cytospin preparations (Diff-Quik; BDH, Poole, U.K.).

Expression of P-selectin on murine endothelial cells

The murine endothelioma cell line, bEnd5 derived from BALB/c mice (Wagner & Risau, 1994), was used to examine P-selectin expression *in vitro*. Cells were cultured to confluence on coverslips in DMEM containing 10% FCS, 10,000 u ml^{-1} penicillin/streptomycin supplemented with 1% non-essential amino acids and 10^{-5} M β-mercaptoethanol. mAb against mouse P-selectin 5H1 and RB40.34, or rat IgG1 as control, were added to the cells to give a final concentration of 10 μg ml^{-1}. The cells were then stimulated with KC (10^{-6} M) or thrombin (30 u ml^{-1}) for 30 min at 37°C. Coverslips were then placed on ice, washed twice and incubated with secondary antibody (Alexa Fluor® 488 goat anti-rat IgG) for 30 min in PBS containing 10% FCS. The coverslips were then washed again and mounted for visualization using a Nikon Eclipse E600 microscope.

Statistical analysis

Results are presented as the mean ± s.e.mean. Data were analysed using one-way analysis of variance (ANOVA) and differences between groups assessed using an appropriate post-test, as indicated. A value of P less than 0.05 was considered significant.

Results

Dose and time dependency of chemokine-induced neutrophil migration into the pleural cavity

The effect of increasing doses of KC on leukocyte recruitment into the mouse pleural cavity was assessed. At 2 h, KC induced a dose-dependent accumulation of neutrophils into the pleural cavity (Figure 1). Total leukocyte numbers in pleural exudates were not significantly increased and the mononuclear cell numbers recovered were not altered with increasing doses of KC (Table 1). Low doses of KC (0.01 and 0.03 μg) significantly increased neutrophil numbers when compared with animals that received intrapleural PBS ($P<0.05$; Figure 1). KC at 0.1 μg per animal resulted in a further significant rise in neutrophils recovered, where the percentage of neutrophils was increased to around 50%. This increase was statistically significant when compared with the lowest dose of KC ($P<0.05$).

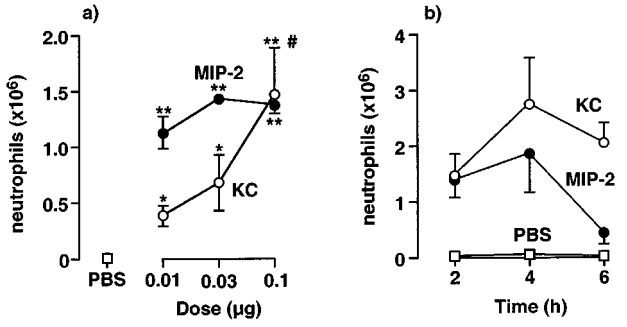

Figure 1 Dose dependency and kinetics of neutrophil accumulation in the pleural cavity in response to KC or MIP-2. (a) increasing doses of KC or MIP-2 (0.01 – 0.1 μg) induced a significant neutrophil accumulation at 2 h when compared with PBS-treated controls. (b) Time course of neutrophil accumulation. Values represent the mean ± s.e.mean of six mice. $^{*}P<0.05$ and $^{**}P<0.01$ compared with PBS, and $^{\#}P<0.05$ compared to KC 0.01 μg by one-way ANOVA followed by Student-Newmann-Keuls post-test.

For comparison, the dose dependency of neutrophil accumulation in response to MIP-2, which is known to act *via* the same receptor as KC, was investigated. MIP-2, at all doses tested, induced a marked recruitment of neutrophils (Figure 1a). Neither total leukocytes nor mononuclear cell numbers were significantly (one-way ANOVA) modified compared with PBS (Table 1). At the lowest doses tested, MIP-2 was more potent than KC but had similar efficacy at the highest dose.

The kinetics of neutrophil recruitment in response to KC and MIP-2 (0.1 μg of each) was also assessed (Figure 1b). At 2 h, similar numbers of neutrophils had migrated. By 4 h, there were fewer neutrophils accumulating in response to MIP-2 than to KC and this pattern was maintained at 6 h with apparent resolution of the response to MIP-2. Total leukocytes recovered from the pleural cavity were elevated at 4 and 6 h when compared with PBS control at the same time but these differences did not achieve statistical significance. There were no differences in mononuclear cell numbers (Table 2). In addition, there was no influx of eosinophils in response to KC or MIP-2 at any of the time points indicating that there was a selective accumulation of neutrophils to both chemokines.

Role of L- and P-selectins

Since L-selectin is expressed constitutively by blood neutrophils, we examined its role in mediating recruitment of neutrophils in response to KC. The anti-L-selectin mAb MEL-14 injected 30 min prior to treatment with KC inhibited neutrophil accumulation at 2 h by 88% ($P<0.01$; Figure 2a). In comparison, pretreatment with the specific anti-P-selectin mAb 5H1, inhibited KC-induced neutrophil accumulation after 2 h by 63% ($P<0.05$; Figure 2a). Co-

Table 1 Dose-dependent effects of CXC chemokines on total leukocytes and mononuclear cell numbers in the pleural cavity

Treatment	*Dose* (μg)	*Total leukocytes* ($\times 10^6$)	*Total mononuclear cells* ($\times 10^6$)
PBS		1.8 ± 0.6	1.8 ± 0.6
KC	0.01	2.5 ± 0.7	2.1 ± 0.7
	0.03	2.2 ± 0.4	1.6 ± 0.2
	0.1	2.6 ± 0.8	1.2 ± 0.3
MIP-2	0.01	3.4 ± 0.4	2.3 ± 0.4
	0.03	3.9 ± 0.9	2.3 ± 0.5
	0.1	2.8 ± 0.5	1.4 ± 0.3

Increasing doses of KC or MIP-2 (0.01 – 0.1 μg per animal) were administered into the pleural cavity and was washed out after 2 h. Total leukocyte counts were determined and mononuclear cell numbers were calculated from differential cell counts. Results are expressed as mean ± s.e.mean of 4 – 6 animals. There were no significant differences in either the total leukocytes or total mononuclear cells recovered between treatment groups (one-way ANOVA).

Table 2 Kinetics of CXC chemokine-induced total leukocyte and mononuclear cell accumulation in the pleural cavity

Treatment	*Time* (h)	*Total leukocytes* ($\times 10^6$)	*Total mononuclear cells* ($\times 10^6$)
PBS	2	1.8 ± 0.6	1.8 ± 0.6
	4	2.1 ± 0.5	1.6 ± 0.5
	6	2.3 ± 0.4	2.2 ± 0.4
KC	2	2.6 ± 0.8	1.2 ± 0.3
	4	5.1 ± 1.5	1.9 ± 0.5
	6	4.1 ± 0.8	2.0 ± 0.4
MIP-2	2	2.8 ± 0.5	1.4 ± 0.3
	4	3.5 ± 0.9	1.6 ± 0.4
	6	1.9 ± 0.3	1.5 ± 0.6

KC or MIP-2 (0.1 μg per animal) was administered into the pleural cavity that was lavaged after 2, 4 or 6 h. Total leukocyte and mononuclear counts were determined. Results are expressed as mean ± s.e.mean of 5 – 6 animals. There were no significant differences in either the total leukocyte or total mononuclear cells recovered between treatment groups (one-way ANOVA).

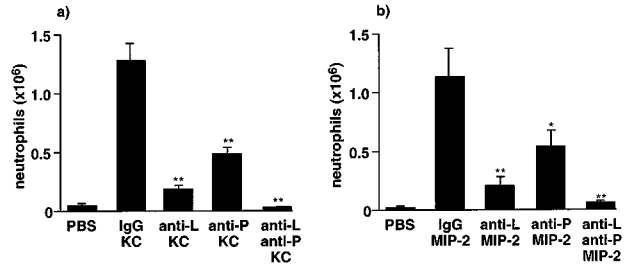

Figure 2 Role of L- and P-selectin in KC- and MIP-2 induced neutrophil accumulation. Mice were treated with an i.v. injection of control IgG, anti-L-selectin alone, anti-P-selectin alone or anti-L- and -P-selectin in combination. KC (a) or MIP-2 (b) was administered (0.1 μg each) into the pleural cavity and neutrophil accumulation measured after 2 h. Values represent mean$\pm$s.e.mean of six mice. $^*P<0.05$ and $^{**}P<0.01$ compared with KC or MIP-2 by one-way ANOVA followed by Student-Newmann-Keuls post-test.

administration of anti-P- and L-selectin mAbs resulted in a further inhibition such that KC-induced neutrophil accumulation was abolished (Figure 2a).

Very similar data was obtained when neutrophil accumulation was induced by MIP-2 (Figure 2b). Thus, responses were reduced 83% by the anti-L-selectin mAb, 53% by anti-P-selectin mAb and 95% by a combination of mAbs.

Circulating neutrophils were not depleted by mAb administration suggesting that the inhibition of accumulation was a result of blocking both selectins. These data indicate that both L- and P-selectin contribute to neutrophil accumulation in response to a preformed chemoattractant and are consistent with a rapid upregulation of endothelial P-selectin by KC and MIP-2.

Role of E-selectin

To determine the role of E-selectin in KC-induced neutrophil accumulation, we examined responses at 2 and 4 h. KC-induced neutrophil accumulation at 2 h ($0.86\pm0.06\times10^6$ neutrophils) and 4 h ($1.79\pm0.18\times10^6$) was unaffected by E-selectin mAb at either time ($1.14\pm0.15\times10^6$ and 1.61×10^6, respectively; mean$\pm$s.e.mean, $n=3$ mice per group). We also examined the effect of blocking P- and E-selectin in combination since our earlier studies using LPS showed that a role for E-selectin was revealed only when P-selectin was also blocked (Henriques *et al*., 1996). Figure 3a shows that blockade of P- and E-selectin, by co-administration of 5H1 and 10E6, did not have an additive inhibitory effect on KC-induced neutrophil accumulation after 2 h (51% reduction) compared with P-selectin blockade alone (see Figure 2a).

We also examined the response after 4 h reasoning that if E-selectin were involved, its contribution may be revealed at this later time. However, as shown in Figure 3b, while P-selectin remains equally important at this time, there is no further reduction in neutrophil accumulation when E-selectin is blocked in combination.

Studies in P-selectin-deficient mice

Blood neutrophil counts in P-selectin +/+ mice were $0.5\pm0.1\times10^6$ ml^{-1} compared with $1.46\pm0.38\times10^6$ ml^{-1} in P-selectin −/− mice. Despite this blood neutrophilia, responses to KC were markedly reduced (by approximately 90%) in P-selectin −/− compared with +/+ when measured after 2 or 4 h (Figure 4). The extent of attenuation in −/− mice was greater than seen in +/+ mice given anti-P-selectin mAb. Responses at both time points were not decreased further in animals given E-selectin mAb but were significantly reduced to the PBS control level in the presence of anti-L-selectin.

Expression of P-selectin on murine endothelial cells

Figure 5 shows that bEnd5 cells express little P-selectin under basal conditions (30 min exposure to PBS alone) compared with cells that were exposed to secondary antibody alone (i.e. no primary antibody). Thrombin induced a marked increase in P-selectin expression consistent with earlier studies showing thrombin could cause degranulation of stored P-selectin and expression on the endothelial cell surface. As seen in Figure 5, exposure to KC also resulted in a rapid increase in cell surface P-selectin expression.

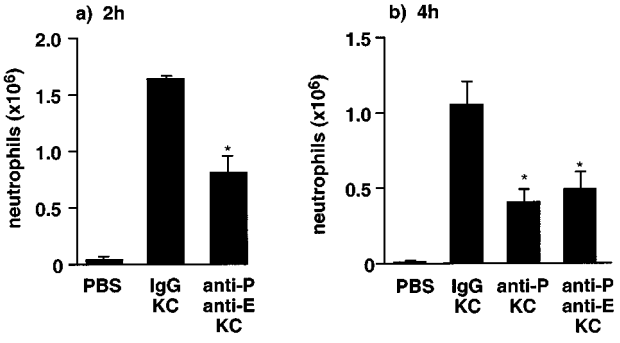

Figure 3 Role of E-selectin in KC-induced neutrophil accumulation. Mice were treated with an i.v. injection of control IgG (non-blocking P- and E-selectin mAbs), anti-P-selectin mAb alone or anti-P- and -E-selectin mAbs in combination. KC (0.1 μg) was administered into the pleural cavity and neutrophil accumulation measured after (a) 2 h or (b) 4 h. Values represent mean$\pm$s.e.mean of 4–6 mice. $^*P<0.05$ compared with KC by one-way ANOVA followed by Student-Newmann-Keuls post-test.

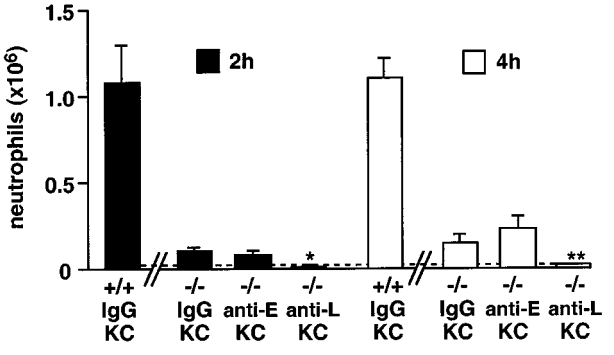

Figure 4 KC-induced neutrophil accumulation in P-selectin −/− mice. P-selectin +/+ or −/− mice were treated with an i.v. injection of control IgG, anti-E-selectin or anti-L-selectin mAbs. KC (0.1 μg) was administered into the pleural cavity and neutrophil accumulation measured after (a) 2 h or (b) 4 h. Values represent mean$\pm$s.e.mean of four mice in each group. The dashed line represents the response to PBS. $^*P<0.05$ and $^{**}P<0.01$ compared with response to KC in −/− mouse by one-way ANOVA followed by Student-Newmann-Keuls post-test.

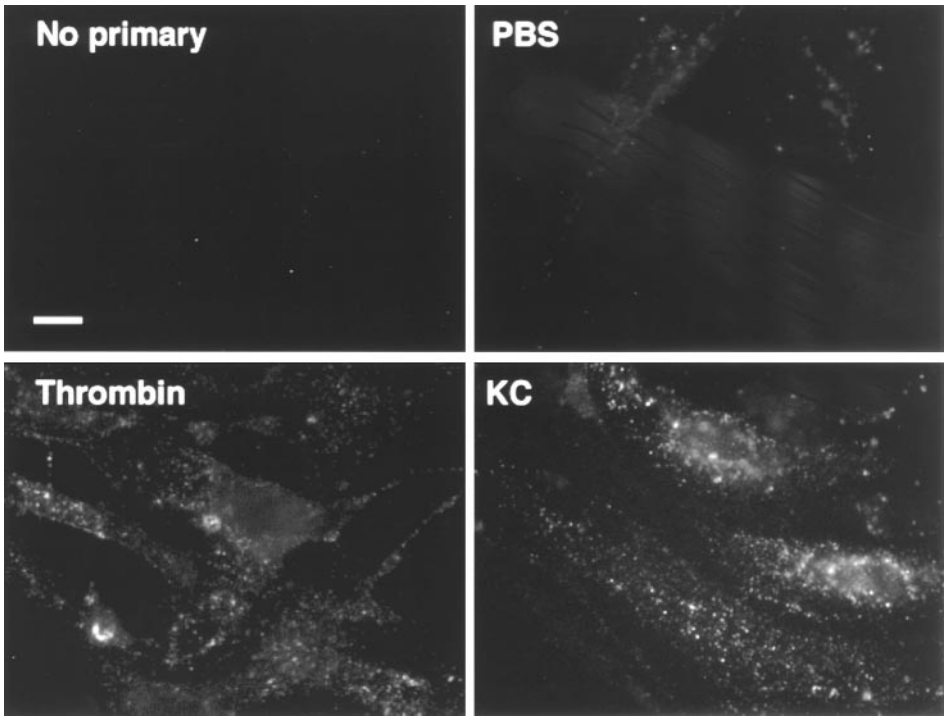

Figure 5 KC-induced P-selectin upregulation on murine endothelial cells *in vitro*. Cultures of the murine endothelioma cell line bEnd5 were treated with PBS without P-selectin mAb (top left), with PBS plus P-selectin mAb (top right), thrombin (30 u ml^{-1}) plus P-selectin mAb (bottom left) or KC (10^{-6} M) plus P-selectin mAb (bottom right). After 30 min, cells were washed and P-selectin expression detected with Alexa Fluor® 488-labelled secondary antibody. The calibration bar represents 20 μm.

Discussion

Our previous work showed that LPS-induced neutrophil accumulation in the mouse pleural cavity was dependent on all three selectins, although inhibition of either endothelial selectin alone was not inhibitory (Henriques *et al*., 1996). LPS induces expression of P- and E-selectin *in vivo* (Gotsch *et al*., 1994) and therefore the requirement for these molecules in mediating neutrophil migration was not unexpected. However, the requirement for selectins in mediating neutrophil accumulation in response to a preformed chemoattractant is less clearly understood. In the present study, we therefore investigated in the pleurisy model the role of selectins in mediating neutrophil migration in response to KC.

In the first part of the study we compared the capacity of KC and a related murine CXC chemokine MIP-2 to induce neutrophil accumulation into the pleural cavity. Both molecules induced a rapid, dose-dependent and selective neutrophil accumulation. While MIP-2 was more potent at the lowest concentrations used, it showed similar potency to KC at the highest dose. This observation differs from that of Tessier *et al*. (1997) who found that MIP-2 was a much more potent and effective inducer than KC of leukocyte accumulation into the mouse air pouch. However, neutrophil numbers were not quantified in their study and our data showed that both these chemokines had little, if any effect on total leukocyte numbers in the pleural cavity. The neutrophil recruiting activity of KC and MIP-2 in the pleural cavity is consistent with previous studies demonstrating the accumulation of neutrophils to these chemokines in other murine organs and tissues (Bozic *et al*., 1995; Wolpe *et al*., 1989).

KC- and MIP-2-induced neutrophil accumulation was dependent on the functional expression of L- and P-selectin, since blockade of these adhesion molecules, separately or in combination, led to a significant impediment of cell emigration. Combined blockade was additive suggesting that P- and L-selectin function largely independently in this model although there was evidence of some overlap since either mAb alone reduced neutrophil accumulation greater than 50%. The role of L-selectin in chemoattractant-induced neutrophil accumulation is consistent with one report showing that LTB_4-induced neutrophil trafficking in mouse skin was attenuated 66% by an L-selectin mAb (Yan *et al*., 1996). Moreover, neutrophil migration to the thioglycollate inflamed peritoneum was reduced by approximately 60% in L-selectin-deficient mice, although the earliest time point studied was 24 h (Tedder *et al*., 1995). In addition, in the same model, Bosse & Vestweber (1994) found that neutrophil accumulation in response to thioglycollate after 2 or 4 h was reduced 60–70% by anti-L- or anti-P-selectin mAbs alone but was abolished by simultaneous blockade of these selectins. Thus, the absolute requirement for L- and P-selectin in neutrophil accumulation is similar when thioglycollate or KC or MIP-2 is used as the stimulus.

In a murine air pouch, administration of CINC (rat homologue of GROα) induced the accumulation of neutrophils (Harris *et al*., 1996). At 2 h, this was reduced 75% by the L-selectin mAb MEL-14 and 59% by the P-selectin mAb 5H1. There was no additional effect of blocking E-selectin in combination with P-selectin although the effect of blocking L- and P-selectin together was not studied. At 4 h, L-selectin mAb was still inhibitory but neutrophil accumulation was no

longer solely dependent on P-selectin, since simultaneous blockade of P- and E-selectin was necessary to abrogate the response. This finding differed from our observation, where simultaneous blockade of P- and E-selectin had no significant effect on neutrophil recruitment at 2 or 4 h. In contrast to the study herein, the air pouch is an artificial cavity induced after 6 days, in which endothelial selectins may be expressed under basal conditions. The pleural cavity has the advantage of being a naive, sterile cavity, consisting of only a single layered mesothelial membrane with a microvascular and lymphatic supply. Thus, while the data of Harris *et al*. (1996) suggest that CINC may induce P-selectin expression in the air pouch microcirculation, it also indicates that E-selectin is upregulated as a result of CINC administration. This could be a direct effect, *via* endotoxin contamination or be a property of the air pouch model.

Intravital microscopic studies have demonstrated a role for P-selectin in the spontaneous leukocyte rolling that is seen after surgery. This surgically-induced P-selectin dependent rolling is thought to occur as a result of mast cell degranulation since treatment with sodium cromoglycate, a mast cell stabilizer, prevents this occurring (Kanwar *et al*., 1995). The observation that antihistamine agents also decrease spontaneous rolling (Ley, 1994) is consistent with an important role of mast cells but also concurs with the known ability of histamine to induce a rapid expression of P-selectin on the surface of cultured human umbilical vein endothelial cells (HUVEC) (Foreman *et al*., 1994). C5a also causes a rapid upregulation of P-selectin on HUVEC but this has not previously been demonstrated for IL-8 or a related molecule. Endothelial cells *in vivo* possess receptors for IL-8 (Rot, 1992) indicating that they have the potential to respond to IL-8 by expressing P-selectin. A possible interpretation of our data is that P-selectin is rapidy upregulated by KC or MIP-2 on post-capillary venules of the parietal and visceral pleura and that this mediates neutrophil rolling. Indeed, we found that KC could increase P-selectin on the murine endothelioma bEnd5. Although these cells are not derived from the parietal or visceral pleura, they are from the microcirculation.

In BALB/c mice, neutrophil accumulation to KC after 2 h was reduced by no more than 70% by anti-P-selectin mAb yet in P-selectin $-/-$ animals, neutrophil accumulation was 90% less than in $+/+$ mice. A possible explanation is diminished L-selectin on P-selectin $-/-$ neutrophils however, as previously reported (Mayadas *et al*., 1993), leukocyte L-selectin is similar in wild type and P-selectin knockouts. Another possibility is that despite using a saturating concentration of mAb there was incomplete suppression of P-selectin function. It has been reported that full P-selectin function requires expression of both lectin and EGF domains (Kansas *et al*., 1994). Monoclonal antibody 5H1 binds to the lectin domain only (Labow *et al*., 1994) so complete blockade of P-selectin function might never be achieved. Finally, it is possible that the requirement for P-selectin in neutrophil accumulation differs between mouse strains.

The results of this study show that neutrophil accumulation into the pleural cavity in response to a direct-acting chemoattractant is mediated by L- and P-selectin with no evidence for a role of E-selectin. Experiments in P-selectin $-/-$ animals indicate that the response is primarily P-selectin dependent with L-selectin playing a smaller role. Based on our studies with a murine endothelioma cell line, we suggest that KC induces the rapid surface expression of P-selectin on the surface of endothelial cells lining the pleural microcirculation. This P-selectin then facilitates neutrophil accumulation by supporting rolling. A P-selectin antagonist, perhaps in combination with an L-selectin blocker, may be an effective anti-inflammatory agent in disease states that are primarily CXC chemokine driven.

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