Participation of Interleukin-5, Interleukin-8 and Leukotriene B_4 in Eosinophil Accumulation in Two Different Experimental Models

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There are several experimental models describing in vivo *eosinophil (EO) migration, including ip injection of a large volume of saline (SAL) or Sephadex beads (SEP). The aim of this study was to investigate the mechanisms involved in the EO migration in these two models. Two consecutive injections of SAL given 48 hr apart, induced a selective recruitment of EO into peritoneal cavity of rats, which peaked 48 hr after the last injection. SEP, when injected ip, promoted EO accumulation in rats. The phenomenom was dose-related and peaked 48 hr after SEP injection. To investigate the mediators involved in this process we showed that BW A4C, MK 886 and dexamethasone (DXA) inhibited the EO migration induced by SAL and SEP. To investigate the source of the EO chemotactic factor we showed that mast cells, macrophages (MO), but not lymphocytes, incubated* in vitro *in presence of SAL released a factor which induced EO migration. With SEP, only mast cells release a factor that induced EO migration, which was inhibited by BW A4C, MK 886 and DXA. Furthermore, the chemotactic activity of SAL-stimulated mast cells was inhibited by antisera against IL-5 and IL-8 (interleukin). SAL-stimulated MO were only inhibited by anti-IL-8 antibodies as well SEP-stimulated mast cells. These results suggest that the EO migration induced by SAL may be dependent on resident mast cells and MO and mediated by LTB_4, IL-5 and IL-8. SEP-induced EO migration was dependent on mast cells and may be mediated by LTB_4 and IL-8. Furthermore, IL-5 and IL-8 induced EO migration, which was also dependent on resident cells and mediated by LTB_4. In conclusion, EO migration induced by SAL is dependent on mast cells and MO, whereas that induced by SEP is dependent on mast cells alone. Stimulated mast cells release LTB_4, IL-5 and IL-8 while MO release LTB_4 and IL-8. The IL-5 and IL-8 release by the SAL or SEP-stimulated resident cells may act in an autocrine fashion, thus potentiating LTB_4 release.*

Key words: interleukin-5 - interleukin-8 - leukotrine B_4 - eosinophil migration - saline - Sephadex

Eosinophils are thought to play an important role in many inflammatory and allergic diseases such as asthma (Barnes et al. 1988, Gleich 1990), atopic dermatitis (Leiferman et al. 1985), allergic rhinitis (Bascon et al. 1989) and parasitic infections (Kay 1985). Although eosinophils are involved in host defense mechanisms against parasites (Capron 1992), they can cause damage to mammalian tissues through a variety of mechanisms, including the release of granule-derived cytotoxic proteins (Gleich et al. 1988) and the generation of toxic oxygen radicals (Davies et al. 1984). Thus, understanding the mediators involved in eosinophil migration may allow us to develop procedures for treating various pathological processes. Several experimental models describing eosinophil migration into the extravascular space have been reported. These models include the injection of polymyxin B (Sun et al. 1985), alum adjuvant (Walls 1977), antigen-coated latex (Schriber & Zucker-Franklin 1974), parasitic larvae or their extracts (Auriault et al. 1983) and large volumes of physiological saline (Cook et al. 1987). However, the endogenous mediators responsible for eosinophil recruitment to the site of inflammation have not yet been fully characterized. Furthermore, intravenous injection of Sephadex has been shown to induce blood and lung eosinophilia in rats and may contribute to bronchial hyperreactivity *in vivo* (in rats) and *ex vivo* (in guinea pigs) (Spicer et al. 1990, Maghni et al. 1993). The mechanisms by which these beads induce eosinophilia also need to be further elucidated.

In addition, several factors including leukotriene B_4 (LTB_4) (Hakansson et al. 1987, Faccioli et al. 1991), $C5_a$ (Ogawa et al. 1981), PAF (Czarnetzki & Csato 1989), interleukin-5

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(IL-5) (Lopez et al. 1988, Sehmi et al. 1992), interleukin-2 (IL-2) (Rand et al. 1991, Meacock et al. 1991), interleukin-8 (IL-8) (Collins et al. 1993), Rantes (Kameyoshi et al. 1992), eotaxin (Jose et al. 1994) and factors derived from mast cells (Holgate 1991, Raible et al. 1992), lymphocytes (Berman & Weller 1992) and macrophages (Lee & Lane 1992) are known to induce eosinophil migration *in vivo* and *in vitro*. LTB_4, IL-5, IL-8 and PAF have been also detected at the site of inflammatory and allergic reactions (Resnick & Weller 1993). However, it is not yet established whether these mediators are direct chemoattractants or act indirectly by stimulating the release of other eosinophil chemotactic factors from resident cells.

The aim of this study was to investigate the mediators involved in eosinophil migration induced by a large volume of saline and by Sephadex beads and the mechanisms by which these mediators induce eosinophil recruitment to the peritoneal cavity of rats.

Figure 1a shows that two consecutive injections of saline (SAL: 0.15 M) at 48 hr intervals induced significant eosinophil migration into the peritoneal cavity of naïve rats 48 hr after the second injection. Eosinophil migration was not influenced by increasing concentrations of sodium chloride (Fig. 1b). Thus, the concentration of SAL used in subsequent experiments was fixed at 0.15 M. In contrast to saline, PBS did not promote eosinophil migration. Therefore, to investigate the mechanism by which saline induced eosinophil migration, we added various ions to SAL in order to reconstitute the PBS ion concentration. The addition of potassium chloride (but not phosphate) blocked the ability of SAL to induce eosinophil migration as shown by the eosinophils/ml of peritoneal wash fluid (means ± SEM): control (PBS), $0.17 \pm 0.05 \times 10^6$; SAL, $1.1 \pm 0.06 \times 10^6$; SAL+ phosphate, $1.4 \pm 0.3 \times 10^6$; SAL + potassium chloride, $0.3 \pm 0.1 \times 10^6$* and SAL+ potassium chloride + phosphate, $0.4 \pm 0.1 \times 10^6$ (*$p < 0.05$, n=6, Student's t-test). A similar inhibition of the ability of SAL to induce the *in vitro* release of a chemotactic factor for eosinophils by mast cells or macrophages (see bellow) was seen by correcting the potassium concentration to the corresponding values for PBS. The mechanism by which SAL induced the *in vitro* release of the eosinophil chemotactic factors or *in vivo* eosinophil migration may involve an alteration of cell membrane properties resulting from a decrease in the potassium ion concentration in the extracellular environment. Potassium chloride is known to play a role in the control of the resting membrane potential (Cook 1988, Janiszewski et al. 1992).

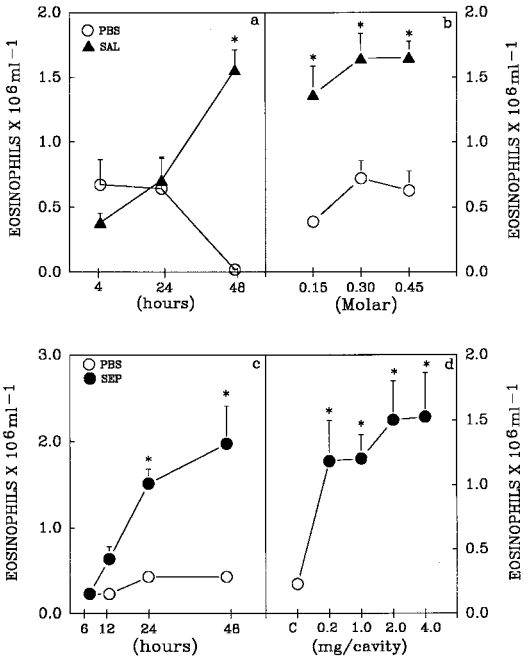

Fig. 1: induction of eosinophil migration by injection of large volumes of saline or Sephadex into the peritoneal cavity of rats. Time-course of the eosinophil migration induced by two consecutive injections of 5 ml PBS (; control), saline (SAL, panel a, S) or a single injection of 2 ml Sephadex (SEP, panel c,) and dose-response curve of eosinophil migration induced by saline (panel b) or Sephadex (panel d) injection into rat peritoneal cavities evaluated 48 hr after the injection of Sephadex or after the last injection of saline. The results are presented as means ± SEM for six animals per group. The asterisk indicates a significant difference between the saline or Sephadex and PBS groups ($p<0.05$; ANOVA followed by Bonfferoni´s t test).

With Sephadex, it was observed that intraperitoneal injection of this stimulus promoted time-dependent eosinophil accumulation (Fig. 1c, d).

To investigate the mediators involved in eosinophil migration induced by saline and Sephadex, the effect of anti-inflammatory drugs on the eosinophil migration induced by both stimuli was evaluated. Pretreatment of the animals with a PAF antagonist (BN 52021, 20 mg/kg/day) or a cyclooxygenase inhibitor (indomethacin, 5 mg/kg/day) had no effect on eosinophil migration while the 5-lipoxygenase inhibitors BW A4C (20 mg/kg/day) and MK 886 (1 mg/kg/day) and the glucocorticoid, dexamethasone (0.5 mg/kg/day) inhibited migration induced by either stimulus (Fig. 2). Thus, PAF-acether and cyclooxygenase products seem not to be involved in SAL or Sephadex-induced eosinophil migration since BN 52021 or indomethacin did not affect the response. The mediator involved in eosinophil migration induced by SAL or Sephadex appears to be a 5-lipoxygenase product since the migration was inhibited by pre-

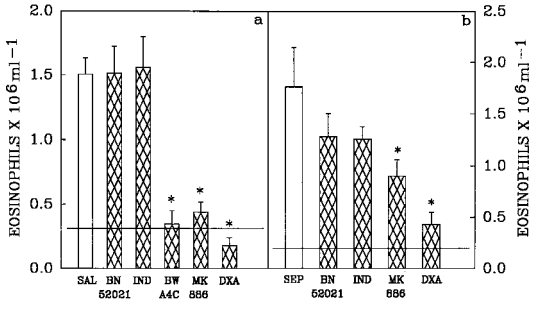

Fig. 2: BW A4C, MK 886 and dexamethasone, but not BN 52021 or indomethacin, inhibited the *in vivo* eosinophil migration induced by saline or Sephadex. The open bar shows the eosinophil migration induced by two injections of saline (SAL, panel a) or Sephadex (SEP, panel b) into PBS-pretreated animals. The hatched bars represent the eosinophil migration in rats pretreated with BN 52021 (20 mg/kg/day), indomethacin (IND, 5 mg/kg/day), BW A4C (20 mg/kg/day), MK 886 (MK, 1 mg/kg/day) and dexamethasone (DXA, 0.5 mg/kg/day). Eosinophil migration was evaluated 48 hr after the injection of SEP or after the last injection of SAL. The dashed line represents the number of eosinophils in rats injected twice with PBS. The results are presented as means ± SEM for six animals per group. The asterisk indicates significant inhibition compared to the response with saline or Sephadex ($p<0.05$; ANOVA followed by Bonfferoni´s t test).

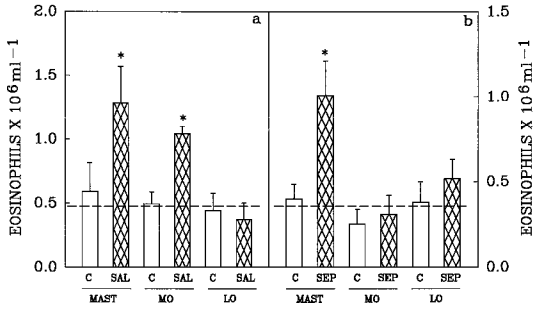

Fig. 3: rat peritoneal resident cells release an eosinophil chemotactic factor when incubated with saline or Sephadex. Mast cells and macrophages, but not lymphocytes, incubated with saline (panel a) released an eosinophil chemotactic factor into the supernatant. Mast cells but not macrophages or lymphocytes incubated with Sephadex (panel b) released an eosinophil chemotactic factor into the supernatant. The supernatants were ultrafiltered and resuspended in the same volume of PBS. Eosinophil migration was evaluated 6 hr after injection of the supernatant. The dashed line represents the number of eosinophils in rats injected with PBS. The results are presented as means ± SEM for six animals per group. The asterisk indicates significant differences in eosinophil migration induced by SAL or SEP and PBS supernatants ($p<0.05$; ANOVA followed by Bonfferoni´s t test).

treatment of the animals with BW A4C and MK 886. LTB_4 is a plausible candidate since it induced eosinophil migration in our model (data not shown), as well as in an *in vitro* assay. In this context, the association between hypersensitivity reactions, the presence of eosinophils and the detection of LTB_4 in lung tissue has been recently suggested.

In order to investigate the role of resident peritoneal cells in SAL- or Sephadex-induced eosinophil migration, isolated peritoneal mast cells, peritoneal macrophages, or lymphocytes collected from the thoracic duct were preincubated with PBS, SAL or Sephadex and the ability of the supernatants to induce eosinophil migration was tested. The supernatants of mast cells and macrophages incubated with SAL, but not with PBS, induced significant eosinophil migration 6, 24 and 48 hr after injection into the peritoneal cavities of naïve rats. In contrast, the supernatant of lymphocytes incubated with SAL was unable to induce eosinophil migration (Fig. 3a). In the Sephadex model, only the supernatant from Sephadex-stimulated mast cells induced significant eosinophil migration when injected into the peritoneal cavities of naïve rats (Fig. 3b). These data suggest that the eosinophil migration induced by SAL is dependent on resident macrophages and/or mast cells whereas that induced by Sephadex only depends on mast cells.

The pretreatment of mast cells or macrophages with BW A4C (100 μM), MK 886 (1 μM) or dexamethasone (10 μM), but not with indomethacin (10 μM) or BN 52021 (100 μM), inhibited the release of the eosinophil chemotactic factor into the supernatants of these cells stimulated by SAL (Fig. 4a, b). Together with the *in vivo* experiments, these results suggest that eosinophil migration induced by saline is mediated by LTB_4, which is released by the resident mast cells and macrophages. In the Sephadex model, the pretreatment of mast cells with MK 886 (1 μM) or dexamethasone (10 μM), but not with indomethacin (10 μM), inhibited the release of the eosinophil chemotactic factor into the supernatant of mast cells stimulated by Sephadex (Fig. 4c). Together with the *in vivo* data, these results support the suggestion that LTB_4 is an important mediator of SAL- or Sephadex-induced eosinophil migration. To investigate whether cytokines are also involved in eosinophil migration induced by SAL or Sephadex, the effect of pretreatment of saline-stimulated mast cell or macrophage supernatants and Sephadex-stimulated mast cell supernatants with control serum or antiserum against IL-1-ß, TNF-α, IL-5 or IL-8 was determined (Fig. 5). Incubation of the supernatants from saline- or Sephadex-stimulated mast cells or macrophages with antibodies against IL-1-ß and TNF-α had no effect on the subsequent eosinophil migration. In contrast, incubation of supernatants from saline-stimulated mast cells with antibodies against IL-5 or IL-8 abolished its ability to induce

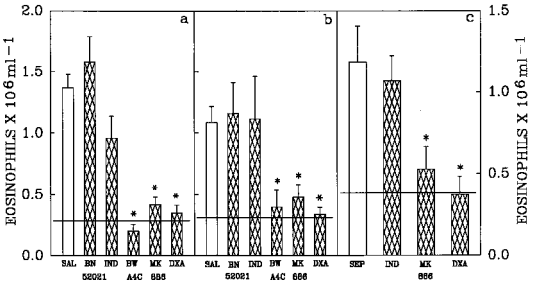

Fig. 4: BW A4C, MK 886 and dexamethasone, but not BN 52021 or indomethacin, inhibited the release of eosinophil chemotactic factor by mast cells and macrophages incubated with saline or by mast cells incubated with sephadex. The bars represent the eosinophil migration induced by the injection of 3 ml of the supernatant of mast cells (a) or macrophages (b) stimulated with saline alone (open bars) or supernatant of mast cells (c) stimulated with Sephadex alone (open bars). The hatched bars represent the cells stimulated with saline or Sephadex plus BN 52021 (100 μM), indomethacin (10 μM), BW A4C (100 μM), MK 886 (1 μM) or dexamethasone (10 μM). The dashed line represents the number of eosinophils after the injection of 3 ml of PBS alone (control). The results are presented as means ± SEM for six animals per group. The asterisk indicates significant differences between the group incubated with saline or Sephadex alone and the groups treated with various drugs ($p<0.05$; ANOVA followed by Bonfferoni´s t test).

eosinophil migration (Fig. 5a). The eosinophil chemotactic activity of supernatants of saline-stimulated macrophages was only inhibited by the antibody to IL-8 (Fig. 5b). In the supernatants from Sephadex-stimulated mast cells only the antibody

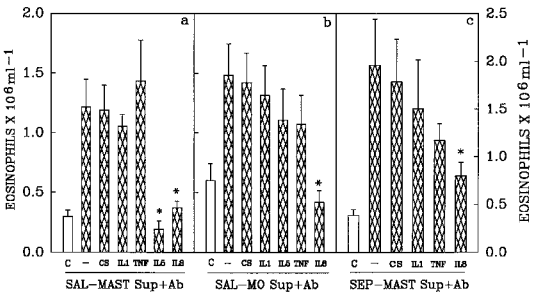

Fig. 5: the effects of antiserum against IL-1, TNF, IL-5 and IL-8 on the eosinophil chemotactic activity of supernatants from mast cells and macrophages incubated with saline or from mast cells incubated with Sephadex. The bars represent the eosinophil migration induced by the injection of the supernatants from mast cells (a) or macrophages (b) previously incubated with saline. Panel c represents the eosinophil migration induced by injection of the supernatant from mast cells incubated with Sephadex. The supernatants were pretreated with PBS (-), control serum (CS) or with IL-1, TNF, IL-5 or IL-8 antiserum before injection. Eosinophil migration was evaluated 6 hr after injection of the supernatants. The asterisks indicate significant differences between the group incubated with PBS (-) and the groups treated with a given antiserum ($p<0.05$; ANOVA followed by Bonfferoni´s t test).

to IL-8 inhibited the eosinophil chemotactic activity (Fig. 5c). All antisera were active at the concentration used since they inhibited the ability of their respective cytokines to induce eosinophil or neutrophil migration into the peritoneal cavity of naïve rats (data not shown). Until now, we have observed that, in addition to LTB_4, mast cells incubated with saline release IL-5 and IL-8, whereas macrophages release IL-8. In the Sephadex model mast cells release LTB_4 and IL-8.

The next question was related to the mechanism which regulated eosinophil migration induced by IL-5 or IL-8. As such, the administration of IL-5 induced a specific and dose-dependent eosinophil migration (3-25 ng/animal), which was already significant 6 hr after cytokine injection, and remained high for up to 24 hr. The dose-response curve induced by IL-8 was bell-shaped. At the dose of 20 ng/rat, significant eosinophil migration was observed, while at doses of 5, 10 and 40 ng/rat the eosinophil migration observed did not differ from that induced by PBS. The migration induced by 20 ng IL-8 only peaked 24 hr after injection of the cytokine and returned to control levels after 48 hr (data not shown). Eosinophil migration induced by IL-5 or IL-8 may have been blocked by the pretreatments of the animals with MK 886 or dexamethasone. The IL-5-induced eosinophil migration was also blocked by BW A4C, another 5-lipoxygenase inhibitor (Fig. 6). We also investigated the role of resident peritoneal cells in IL-5 or IL-8-induced eosinophil migration. Eosinophil migration induced by IL-5 or IL-8 was inhibited by 85 and 80%, respectively, following

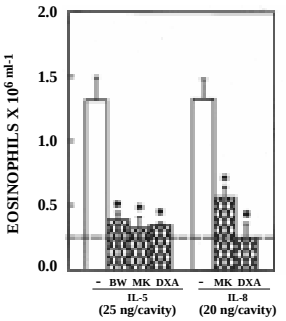

Fig. 6: BW A4C, MK 886 and dexamethasone inhibited the eosinophil migration induced by IL-5 or IL-8. The open bars show the eosinophil migration induced by IL-5 (25 ng/cavity) or IL-8 (20 ng/cavity) in PBS-pretreated animals. The hatched bars represent the eosinophil migration in rats pretreated with MK 886 (MK, 1 mg/kg), BW A4C (BW, 20 mg/ kg) or dexamethasone (DXA, 0.5 mg/kg). Eosinophil migration was evaluated 24 hr after the injection of IL-5 or IL-8. The dashed line represents the number of eosinophils in rats injected twice with PBS. The asterisks indicate significant inhibition compared to the response in the nontreated groups (-) ($p<0.05$; ANOVA followed by Bonfferoni´s t test).

prior depletion of the all resident cells by lavage of the peritoneal cavity (data not shown). These data suggest that the eosinophil migration induced by IL-5 or IL-8 is also dependent on the resident peritoneal cells.

In conclusion, our results indicate that the eosinophil migration induced by saline is dependent on resident mast cells and macrophages, whereas that induced by Sephadex is only dependent on mast cells. Stimulated mast cells release LTB_4, IL-5 and IL-8 and macrophages release LTB_4 and IL-8. IL-5 and IL-8 released by the saline- or Sephadex-stimulated resident cells may act in an autocrine fashion, thus potentiating the LTB_4 release.

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