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Eotaxin levels and eosinophils in guinea pig broncho-alveolar lavage fluid are increased at the onset of a viral respiratory infection

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Summary

In previous studies we found that guinea pigs demonstrate an increase in airway reactivity and eosinophil numbers 4 days after a respiratory infection with parainfluenza-3 (PI3) virus. Clinical data support the possible involvement of eosinophils in virus-induced airway hyperresponsiveness. Eotaxin, a newly discovered chemokine, could be involved in eosinophil migration to the airways. In this study, eosinophil numbers were counted in blood and bronchoalveolar lavage (BAL) fluid and related with eotaxin concentrations in BAL fluid 1, 2, 3, and 4 days after intratracheal PI3 virus administration. On day 1, blood eosinophils increased by more than 200% ($P < 0.01$). The number of eosinophils were only slightly enhanced from day 2 to day 4 (40%–70%). BAL fluid eosinophils were not increased on day 1 but were significantly elevated on day 2 (180%) and remained high on days 3–4 (>300%, $P < 0.05$). This increase in lung eosinophils correlated well with eotaxin levels measured in BAL fluid. There was no significant increase in eotaxin on day 1 following PI3 infection; however, on days 2–4 eotaxin levels in BAL fluid were significantly elevated (four–sixfold increase) when compared with medium inoculated controls. Eotaxin appears to play an important role in eosinophil accumulation in guinea pig lung following PI3 infection.

Keywords: asthma, eotaxin, viral infections

Introduction

Viral respiratory infections can cause airway hyperresponsiveness in healthy humans and cause exacerbations in asthma patients [1]. It has been reported that 80% of patients with asthma exacerbations tested positive for viral respiratory infections [2].

Experimental infection with respiratory viruses in humans results in an increased airway responsiveness, increased submucosal lymphocytes, and epithelial eosinophils, which seem to persist even after convalescence [3]. A correlation exists between changes in PC 20 and changes in the number and activation of eosinophils in sputum in asthma patients experimentally infected with a respiratory virus [4]. Therefore, there might be a relationship between viral respiratory infections, activation of inflammatory cells, and airway responsiveness.

In our laboratory an animal model was developed in which guinea pigs were infected intratracheally with parainfluenza-3 (PI3) virus. Four days after the viral infection, guinea pigs expressed airway hyperresponsiveness to histamine both *in vitro* and *in vivo* [5,6]. Also, the number of bronchoalveolar lavage (BAL) cells, especially eosinophils, was increased [7]. The airway eosinophilia and airway hyperresponsiveness was still present 8 and 16 days after infection [6].

Chemokines are involved in the control of leucocyte migration [8]. Recently, the C-C chemokine eotaxin, discovered

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in BAL of guinea pigs sensitized and challenged with ovalbumin, was found to be a potent eosinophil attractant [9]. Eotaxin induces a selective accumulation of eosinophils when administered to the airways or injected intradermally [10,11]. The receptor for eotaxin, CCR-3, is expressed in high numbers on eosinophils [12] and also on basophils and Th2 cells [13,14]. The role of eotaxin in the development of inflammation during allergic reactions is extensively studied. Eotaxin expression correlates with eosinophil influx in sensitized guinea pigs challenged with ovalbumin [15]. Moreover, an increase in eotaxin levels in BAL fluid from asthmatics has been observed [16].

In this study the role of eotaxin in a time course study of airway eosinophilic influx in guinea pigs treated with PI3 virus was investigated. Migration of eosinophils from the blood to the airways was detected and was related to eotaxin levels in BAL fluid.

Methods

Animals

Specified-pathogen-free male Dunkin-Hartley guinea pigs (350–450 g, Harlan Olac Ltd, UK) were housed under controlled conditions in cages under filter-tops. Commercial chow and water were supplied *ad libitum*.

Viral infection

Animals were treated with PI3 virus as described before [6]. The animals were anaesthetized with ether. Thereafter, 0.1 mL of the virus suspension (ID-DLO, Lelystad, The Netherlands, tissue culture invective $dose_{50} = 10^{8.9}$/mL) was administered intratracheally. Growth medium was subjected to a similar procedure in order to serve as a control solution.

Bronchoalveolar lavage

Animals were killed 1, 2, 3, or 4 days after virus inoculation by an overdose of Euthesate (sodium pentobarbital, 300 mg/kg). BAL was performed as described previously [5]. From the first 10 mL, only 7 mL was retrieved after 1 min, and stored in a plastic tube on ice. After centrifugation, 1 mL of cell-free supernatant was stored for measurement of guinea pig eotaxin with a radio immuno assay as previously described [15]. After recovery of the first 7 mL, the lungs were lavaged three times with 10 mL saline. The cells were sedimented by centrifugation at 580 ***g*** for 10 min at 4 °C. The cells were resuspended in 1 mL saline and cytospins were prepared. Cytospin preparations were stained with Diff Quick and the cells enumerated.

Table 1. Percentage of eosinophils in blood and BAL on days 1, 2, 3, and 4 after intratracheal administration of PI3 virus (mean ± SEM, $n = 3$) or medium-treated animals (mean ± SEM of four animals, $n = 4$)

	Eosinophils (%)	
	Blood	BAL
Medium	17.5 ± 2.6	11.0 ± 1.1
Virus day 1	54.5 ± 6.1*	11.0 ± 2.3
Virus day 2	29.2 ± 4.0	31.7 ± 24.4**
Virus day 3	25.5 ± 4.9	47.5 ± 14.3**
Virus day 4	24.0 ± 5.9	42.9 ± 15**

*$P < 0.01$, **$P < 0.05$, compared with medium-treated animals.

Blood cell differentiation

Blood was obtained by a cardiac puncture. Blood smears were stained with Diff Quick and leucocytes enumerated.

Results

Blood and BAL analysis

Blood and BAL cells were differentiated 1, 2, 3, and 4 days after treatment with PI3 virus (Table 1). One day after virus administration, the number of eosinophils in peripheral blood was significantly increased by more than 200% ($P < 0.01$). The percentage of blood eosinophils 2, 3, and 4 days after viral infection was slightly increased when compared with medium-treated guinea pigs. In BAL fluid

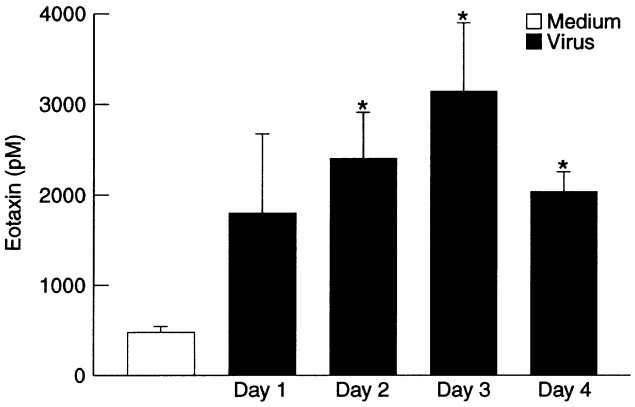

Fig. 1. Eotaxin concentrations in BAL fluid of guinea pigs on days 1, 2, 3, or 4 after intratracheal administration of PI3 virus (black bars) and control-treated animals (white bar). Results are expressed as mean ± SEM ($n = 3$–4). Asterisks indicate $P < 0.05$ compared with medium-treated animals.

a different pattern of eosinophil numbers was detected (Table 1). In contrast to the blood eosinophilia observed at day 1 following PI3 infection, BAL fluid eosinophils were not increased at this time. On day 2 following PI3 virus infection, the percentage of eosinophils increased by more than 180% and remained significantly elevated on days 3 and 4 (>300%, $P < 0.05$).

Eotaxin concentrations in BAL

Eotaxin concentrations were measured in BAL fluid of control and virus-treated guinea pigs (Fig. 1). On day 1 eotaxin levels started to increase. There was a 4–6 times increase in eotaxin concentrations on days 2–4 ($P < 0.05$) after viral infection compared with medium-treated animals.

Discussion

Infection of guinea pigs with PI3 virus results in airway hyperresponsiveness and an influx of inflammatory cells, mainly eosinophils [5]. The eosinophil influx in the lung and the degree of airway hyperresponsiveness are associated [7,17].

From the time course study on peripheral blood eosinophils it appeared that during the virus infection there was a marked blood eosinophilia on day 1. Two days after infection, eosinophil numbers were not significantly different from those observed in control guinea pigs. The increase in BAL fluid eosinophils was observed at a later time than that seen with blood eosinophils. Two days after the viral infection eosinophil numbers started to increase. The levels remained significantly elevated up until 4 days after infection. These results point to a migration of eosinophils from the blood to the lungs. The increased number of eosinophils in the lung coincides with an increase in eotaxin levels in BAL fluid. This suggests that eotaxin might be involved in the attraction of blood eosinophils to the lungs.

Airway hyperresponsiveness in virus-treated guinea pigs was decreased after treatment with antibodies to IL-5; however, at the highest dose of histamine a residual increase in airway response was still present both *in vitro* and *in vivo* [18]. IL-5 has been shown to play an important role in eosinophil recruitment in many animal models of inflammation. In this virus model, however, treatment with antibodies to IL-5 had almost no effect on the eosinophil influx into the lungs, but blood eosinophils dropped significantly [18].

Since the increased eotaxin concentrations coincide with the influx of eosinophils to the lungs, it is hypothesized that eotaxin might be involved in migration rather than IL-5. IL-5 has been shown to have several actions on eosinophils. It proliferates and differentiates bone marrow eosinophils, it prolongs eosinophil survival, and it primes eosinophils to respond better to other stimuli. Cooperation between IL-5 and eotaxin has been observed in many studies. Both *in vitro* and *in vivo* studies indicate that after treatment with IL-5 eosinophils respond better to eotaxin. *In vitro* IL-5 enhances eosinophil migration to eotaxin [19]. In guinea pigs, eotaxin is able to induce tissue eosinophilia after injection into the skin; however, this accumulation of eosinophils is more pronounced when the animals are pretreated with IL-5 [11]. Since antibodies to IL-5 in virus-treated animals have only a small effect on eosinophil migration to the lungs [18], it might be possible that in this model there is cooperation between IL-5 and eotaxin.

From the data mentioned above we hypothesize that due to the inflammatory response induced by the viral infection, IL-5 might be involved in an increase in eosinophils in the circulation. Epithelial cells, endothelial cells, mast cells, and macrophages might be triggered to produce eotaxin. In response to eotaxin, the circulating eosinophils will adhere to the blood vessels and eventually migrate to the site of eotaxin production.

Eotaxin appears to play a pivotal role in eosinophil recruitment and eotaxin concentrations have shown to be increased in asthma patients [16]. Therefore, chemokines might be an attractive therapeutic target for the treatment of diseases characterized by eosinophilic inflammation.

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