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会長推薦講演 I

PATHOGENESIS OF PLATELET ACTIVATING FACTOR-INDUCED INFLAMMATION IN THE AIRWAY MUCOSA

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Introduction

Platelet activating factor (PAF), a highly potent inflammatory mediator formed by the action of phospholipase A_2 on membrane phospholipids, constricts the airway smooth muscle, induces microvascular leakage in several tissues, causes the aggregation of inflammatory cells including eosinophils and damages the airway epithelium[1]. As it becomes apparent that no single mediator or inflammatory cell can account for allergic inflammation, increasing attention has been paid to PAF as a mediator of allergic inflammation. However, the mechanisms of PAF-induced inflammatory reactions are not yet clearly defined.

Nitric Oxide Mediates PAF-Induced Microvascular Leakage in Rat Airways

Nitric oxide (NO), first identified as endothelium-derived relaxing factor, acts as a signal molecule in a variety of important biological systems[2]. The possible involvement of NO as a component of airway microvascular leakage has been suggested[3]. However, the role of NO in PAF-induced microvascular leakage in airways is not yet established.

To investigate the role of NO in PAF-induced microvascular leakage in rat nasal mucosa and trachea, PAF (1 μg/kg) was injected intravenously, and the amount of PAF-induce microvascular leakage was measured with extravasation of Evans blue dye (30 mg/kg, intravenously injected 5 minutes before the injection of PAF) using spectrophotometry and fluorescence microscopy, and the inhibition of PAF-induced microvascular leakage was determined after pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg, intravenously injected 1 hour before the injection of PAF, n=5) to inhibit the NO synthase (NOS), while the control rats (n=4) were pretreated with normal saline.

The average amount of extravasated Evans blue dye is significantly inhibited in L-NAME pretreated rats than in control rats (t test, $p<0.01$, Fig. 1). Tissue sections of the L-NAME-pretreated rat clearly showed a decreased extravasation of Evans blue dye under the fluorescence microscopy.

The role of NO in modulating microvascular perme-

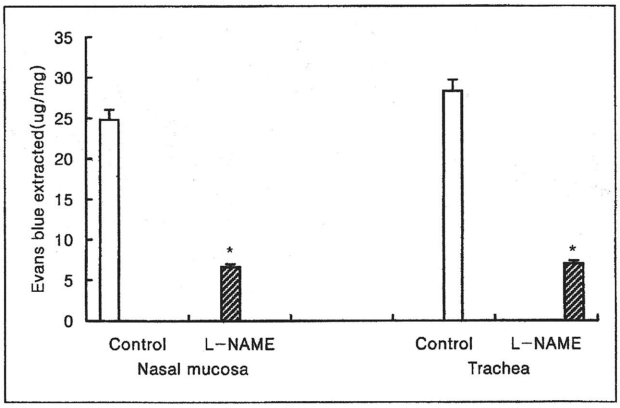

Fig. 1 The amount of PAF-induced extravasated Evans blue dye in the nasal mucosa and trachea. Extravasated Evans blue dye is significantly inhibited in L-NAME pretreated rats than in control rats. (* : $p<0.01$)

ability is still controversial. Endogenous NO suppresses plasma leakage under physiological conditions[4]. But under certain pathological conditions, induced with SP, LD4 or allergen, the activated endogenous NO increases plasma leakage in airway microvessels[5]. Our findings imply that PAF also may activate endogenous NO to increase plasma leakage in airway microvessels.

NOS exists in several isoforms. The neuronal and endothelial NOS are constitutive (cNOS), and the inducible NOS (iNOS) is not constitutively expressed but induced by certain stimuli. De novo synthesis of iNOS requires about 2 to 4 hours from the time of induction[6]. L-NAME inhibits both cNOS and iNOS. In this study, PAF-induced microvascular leakage occurs on the relatively short time interval, within 5 minutes. Therefore, L-NAME appears to inhibit cNOS rather than iNOS in our experimental conditions. Our findings imply that PAF may activate the cNOS in the endothelium, and the activated endogenous NO may mediate PAF-induced microvascular leakage in rat airways.

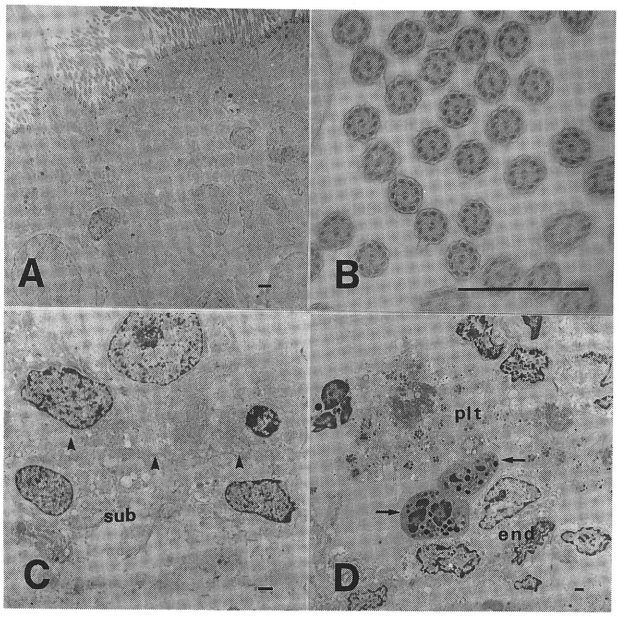

Fig. 2 Electron micrographs of the rabbit sinus mucosa 1 day after administration of PAF. The epithelial cells and cilia show no ultrastructural changes (A, B). Subepithelial space (sub) shows no evidence of infiltration of inflammatory cells (C). Platelet (plt) aggregation, red blood cell stasis and swelling of the endothelial cells (end) are observed (D). (arrowheads=basement membrane, arrows=eosinophil, bars=1μm)

Ultrastructural Changes in PAF-Induced Epithelial Damage in the Rabbit Maxillary Sinus Mucosa

PAF has been known to induce mucociliary inhibition and epithelial damage to the airway mucosa[7]. However, several recent papers have reported that PAF may not readily damage the airway epithelium[8].

To elucidate the pathogenesis of PAF-induced epithelial damage, we administered PAF (16 μg/ml) into the rabbit maxillary sinuses in vivo, and investigated the ultrastructural changes in the sinus mucosa, according to time intervals, 1 and 3 days (n=3 in each) after administration of PAF.

No epithelial degeneration was observed other than platelet aggregation, red blood cell stasis, and swell-

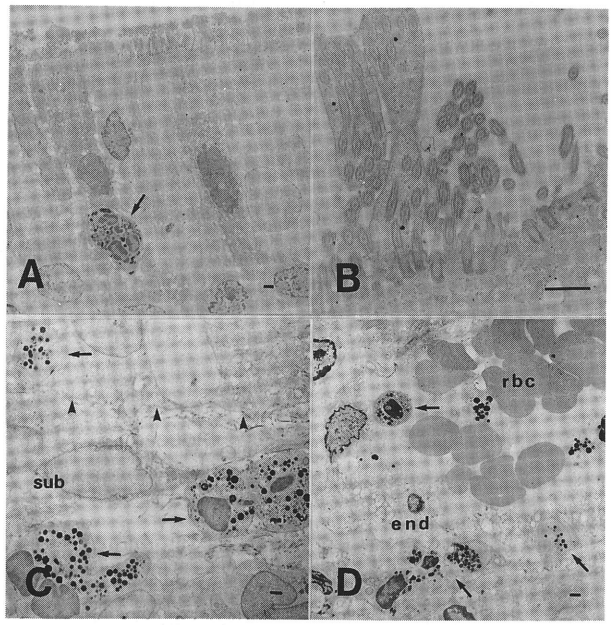

Fig. 3 Electron micrographs of the rabbit sinus mucosa 3 days after administration of PAF. The epithelial cells show vacuolar degeneration with fusion or focal loss of cilia. An eosinophil (arrow) infiltrated into intraepithelial space is observed (A, B). Eosinophils (arrows) are infiltrated into subepithelial space (sub) (C). Swelling of the endothelial cells (end) and migration of eosinophils (arrows) into the perivascular connective tissue are observed (D). (arrowheads=basement membrane, rbc=red bloodcell, bars=1μm)

ing of the endothelial cells 1 day after administration of PAF (Fig. 2). Migration of inflammatory cells into the perivascular connective tissue, infiltration of eosinophils into subepithelial and intraepithelial spaces, and vacuolar degeneration of the epithelial cells with focal loss of cilia were seen 3 days after administration of PAF (Fig. 3).

PAF is one of chemotactic mediators for various inflammatory cells. Of particular interest is the potent chemotaxis of PAF for eosinophils[1]. It has been suggested that PAF-activated eosinophils may release cytotoxic mediators to disrupt the epithelium[9]. Our findings according to time intervals suggest that PAF may cause epithelial damage through a series of secondary events, probably by the cytotoxicity of migrated eosinophils into the epithelium.

Conclusion

Pretreatment with L-NAME clearly inhibited PAF-induced microvascular leakage in the nasal mucosa and trachea of the rat. This finding implies that PAF may activate the cNOS in the endothelium, and the activated endogenous NO may mediate PAF-induced microvascular leakage in rat airways.

PAF induced infiltration of eosinophils in the epithelium, and then resulted in epithelial degeneration. Our findings suggest that PAF may cause epithelial damage through a series of secondary events, probably by cytotoxicity of eosinophils infiltrated into the epithelium.

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