Cigarette Smoke-induced Acute Eosinophilic Pneumonia Accompanied with Neutrophilia in the Blood

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Abstract

Smoking causes various changes in the lung. This report describes a case of cigarette smoke-induced acute eosinophilic pneumonia (CS-AEP) with neutrophilia in the blood. However, the precise mechanism is unknown, so we examined the effect of exposure to cigarette smoke extracts on the production of interleukin (IL)-4, IL-5, IL-8, IL-18, granulocyte macrophage-colony stimulating factor (GM-CSF), and vascular endothelial growth factor (VEGF) by human bronchial epithelial cells (HBECs) obtained from the patient. We found that IL-8 released from HBECs was involved in neutrophilia in the blood, and is a new factor in the development of AEP, especially in the early phase. (Internal Medicine 41: 993-996, 2002)

Key words: cigarette smoke extract, interleukin 8, bronchial epithelial cell

Introduction

Acute eosinophilic pneumonia (AEP) was first described in 1989 (1), and there have been recent reports on cigarette smoke-induced AEP (CS-AEP) (2). Interestingly, circulating neutrophilia is usually found during the early phase of AEP (3, 4), although the precise mechanism remains to be elucidated. We outline a case of CS-AEP accompanied with neutrophilia in the blood and examined whether or not CS stimulated cytokine production in human bronchial epithelial cells (HBECs) in this case.

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Case Report

A 16-year-old woman started smoking several cigarettes (HOPE®) per day 1 mo prior to admission and changed to another brand (Marlboro menthol®) 3 weeks before admission. On October 23, 2000, she experienced sudden chest pain and fever (39°C), and was admitted to our hospital on October 25. Chest CT scan demonstrated patchy infiltrates predominantly in the lower field of both lungs with prominence around the bronchi. The following laboratory data were obtained: Arterial blood gas analysis revealed Pao2 64.8 mmHg with oxygen by nasal cannula, 5 l/min. Peripheral blood examination showed a WBC count of 15,000/μl, with a differential cell count of 92% neutrophils, without eosinophils. IL-5 and IL-8 in peripheral blood were 1,831 pg/ml and 24 pg/ml, respectively. The results of serum mycoplasma, chlamydia, aspergillus, and viral antibody tests and bacterial examinations were all negative. A bronchoalveolar lavage (BAL) fluid sample obtained on day 2 included an abundance of leukocytes with 25% eosinophils which had nuclear hypersegmentation (three nuclei, 13%). Transbronchial biopsies were consistent with eosinophilic pneumonia. Steroid pulse therapy was started on day 2 (methylprednisolone sodium succinate, 500 mg/day×3 days). On day 5, the WBC count was 5,800/μl with 18% eosinophils without an increase in neutrophils (30%), and chest X-ray film and IL-5 level in peripheral blood showed marked improvement. From these findings, this case was diagnosed as AEP, consistent with the diagnostic criteria of Allen and Davis (5). Lymphocyte-stimulating tests using cigarettes (HOPE® and Marlboro menthol®) were negative. After discharge, the patient resumed smoking cigarettes other than Marlboro menthol® without any symptoms.

Effect of exposure to CS on HBECs

HBECs were obtained from this case in December 25, 2000 and a volunteer after obtaining informed consent. The volunteer was a non-smoker with normal pulmonary function. The collection and culture of HBECs were performed as previously
Miki et al. described (6). First, 10 times-brushings (Model BC-15C, Olympus) to collect HBECs were performed from the subsegmental bronchi with 10 gentle strokes on the epithelial surface via bronchoscopy. The cells obtained by brushing were collected in a 50-ml polypropylene tube containing 20 ml RPMI 1640 with gentamicin by shaking the brush in the medium. The cells were then washed twice by centrifugation at 400 x g at 4°C for 10 minutes. The rinsed pellet of cells obtained by brushing was resuspended in LHC9/RPMI 1640 medium. We plated aliquots of HBECs in LHC9/RPMI 1640 medium on 24-well culture plates, and incubated the plates until the cells had grown to confluency (Primary culture). The medium was changed after 24 hours and then every 48 hours. The BEAS-2B cell line (human bronchial epithelial cell line) was purchased from the American Type Culture Collection (Manassas, VA) and cultured as recommended by the manufacturer. Then, exposure of HBECs and BEAS-2B cells to a CS extract (CSE) were carried out by a modification of the method previously described (7). Briefly, CSE was generated by respectively burning a HOPE® or Marlboro menthol® cigarette without a filter, and leading the smoke stream (60 ml) into a syringe. The CS was then repeatedly bubbled ten times through another syringe containing 20 ml RPMI 1640. The resulting suspension was adjusted to pH 7.4 with concentrated NaOH and filtered through a 0.45-µm filter (Millipore Corp., Bedford, MA). HBECs and BEAS-2B cells were exposed to the medium containing the 10% CSE-suspension for 6 hours or 24 hours. After these exposures, the culture supernatants were measured with enzyme-linked immunosorbent assays (ELISAs) for interleukin (IL)-4, IL-5, IL-8, IL-18, vascular endothelial growth factor (VEGF), and granulocyte macrophage-colony stimulating factor (GM-CSF), as described previously (8).

Results showed that only the exposure of HBECs, which were obtained from this patient, to CSE from Marlboro menthol® increased the production of IL-8 in a time-dependent manner. In contrast, exposure of HBECs obtained from this patient to CSE from HOPE® did not increase the production of IL-8 (Fig. 1A, top panel). The exposure of HBECs in the normal volunteer and BEAS-2B cells to CSE from either Marlboro menthol® or HOPE® led to no significant increased release of IL-8 (Fig. 1A, bottom panel and 1B). These findings indicated that the significant increase of IL-8 production induced by Marlboro menthol® was a specific phenomenon in this AEP patient. In addition, six hours and 24 hours exposure of HBECs, obtained from this patient, to CSE from either Marlboro menthol® or HOPE® had no influence on the production of other cytokines (IL-4, IL-5, and IL-18), GM-CSF, or VEGF (data not shown).

Since endotoxin is known to stimulate IL-8 production from HBECs, we examined whether the concentration of endotoxin affected the differential effect of CSE from 2 cigarettes on IL-8 production in this study. We measured endotoxin in the CSEs (HOPE® and Marlboro menthol®) and only RPMI 1640 without CSE using a kinetic turbidimetric assay. The concentrations of endotoxin were as follows: HOPE®, 3.8±0.1 pg/ml; Marlboro menthol®, 2.2±1.1 pg/ml; only RPMI 1640 without CSE, 2.0±0.6 pg/ml (n=2). From these data we concluded that the effect of the endotoxin was not important.

### Discussion

Smoking causes various pathophysiologic changes in the respiratory system and is associated with profound consequences on respiratory health. In Japan, some reports have demonstrated CS-AEP (2-4). Herein, we described a case of menthol type cigarette smoking-induced AEP accompanied by neutrophilia in the blood. Cigarette smoke has the capacity to damage the bronchi in a number of ways, including direct toxicity...
to the bronchial epithelium, oxidative damage, and recruitment of inflammatory cells (9). Although the mechanisms by which the smoking, composed of more than 4,000 elements including menthol (9), affects the incidence or severity of AEP are completely unknown, it has been reported that eosinophils represent the major cell population contributing to the inflammatory reaction associated with AEP (1–5). Generally, most eosinophils have two nuclei. Various chemotactic agents induce nuclear hypersegmentation in eosinophils (10). In our case, eosinophils with hypersegmented nuclei in BAL were clearly found. Furthermore, elevated IL-5, which was seen in our case, may initiate the recruitment of eosinophils, increase eosinophil survival, and enhance the release of mediators from eosinophils (11, 12). In recent years, the level of eotaxin, a potent and eosinophil-specific chemoattractant, was found to be high in patients with eosinophilic pneumonia (EP) and its level is significantly correlated with the number of eosinophils in BAL, suggesting that eotaxin, as well as IL-5, plays an important role in lung eosinophilia in patients with EP, although we did not measure its concentration in this study (13). Furthermore, IL-5-stimulated eosinophils responds to IL-8 because IL-8 receptors were undetectable on freshly isolated eosinophils, but were detectable after priming with IL-5 in vitro (14). Thus, much attention has been paid to cytokines, such as the well-studied neutrophil chemoattractant IL-8 on allergic inflammation (14–16).

On the other hand, circulating neutrophilia was followed by circulating eosinophilia in the early phase in AEP in most cases reported to date (3, 4), as well as in the present case. However, the triggering mechanisms for AEP are entirely unknown. Because HBECs are the first line of defense against CS, the differential phenomenon of HBECs to CSE from 2 cigarettes, especially Marlboro menthol®, may explain the mechanisms. Therefore, we examined the effect of exposure to CSE on production of the above factors by HBECs. The finding that the level of IL-8 was high in the culture supernatants of HBECs after exposure to CSE from Marlboro menthol® in this case, but not in peripheral blood on admission, probably indicates that IL-8 production in the lung exposed to Marlboro menthol® was increased by a local immune response. This may be partially due to the period of blood collection for IL-8 measurement. We may have found a high concentration of IL-8 if the patient had visited our hospital earlier. In another case of menthol-type cigarette smoking-induced AEP accompanied with neutrophilia in the blood in our hospital, we found a high concentration of IL-8 in peripheral blood before appearance of patchy infiltrates in chest X-ray film in the early phase of AEP (detailed data not shown). IL-8 and neutrophils are known to be important in airway inflammation associated with cigarette smoke (17–20). BALF specimens from nonasthmatic smokers have greater concentrations of IL-8 and neutrophils than nonsmoker, and have no elevated concentration of eosinophils (17). In this case, evidence that the effect of CS from Marlboro menthol® on IL-8 production is specific phenomenon can be seen in the findings of our study. Interestingly, a previous study indicated a significant correlation between IL-8 levels and the number of neutrophils, but not that of eosinophils in BAL of patients with EP (13). Therefore, it is possible that IL-8, which was produced in the lung locally, has a closer connection with neutrophils rather than eosinophils in the early phase of AEP, although a recent report suggest that IL-5 primed the eosinophil response to IL-8 (14). In addition, these findings suggest that locally produced IL-8 from HBECs may play important roles in the pathophysiology of AEP by inducing circulating neutrophilia in the early phase in AEP.

In summary, although further analyses and larger studies are required to elucidate the role of IL-8 in AEP, we suggested for the first time the possibility that the production of IL-8 released from HBECs is associated with an increased number of neutrophils, and that HBECs play a role in the pathogenesis of CS-AEP by releasing IL-8, especially in the early phase.

**References**


