

Bronchial and Basophil Reaction to Polymyxin B in Asthmatic Patients

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The hypothesis that increased reactivity to polymyxin B in asthmatic patients is not limited to the bronchi but also exists in basophils was investigated in 26 atopic asthmatic subjects. Quantification of basophil degranulation induced by polymyxin B was performed using a flow-cytometric method. Significant degranulation was noted in 17 patients, but in none of 10 healthy controls. There was close relationship between basophil "releasability" to polymyxin B and bronchial reactivity to inhaled polymyxin B. These findings support the hypothesis.

Key words: basophil degranulation; bronchoconstriction; polymyxin B inhalation.

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Polymyxin B, a polypeptide antibiotic, is often used as a potent agent for degranulating mast cells *in vitro* (6). It is known that bronchospasm may occur in patients with chronic obstructive lung diseases after therapeutic inhalation of polymyxin B (2, 11). As atopic asthmatic patients are especially susceptible to this agent, it has been used to detect the allergic background of bronchial obstruction (4). One may assume that polymyxin B induces airway obstruction via degranulation of mast cells and subsequent release of anaphylactic mediators. To verify this hypothesis we examined blood basophils of asthmatic patients for susceptibility to polymyxin B.

MATERIAL AND METHODS

Clinical evaluation

26 patients with mild atopic asthma and 10 healthy controls participated in the study. The patients were evaluated according to medical history (seasonal anamnesis) and positive skin

prick tests to pollens (Bencard). Many of the patients also had positive prick test to other common allergens such as house dust, feathers, moulds. They had not undergone hyposensitization therapy in the last 2 years or treatment with disodium cromoglycate in the 4 weeks prior to the study.

Inhalation of polymyxin B

The patients inhaled 1 ml polymyxin B sulphate in saline at a concentration of 20 mg/ml. The aerosol was generated in a Wisbadener glass inhalator with an oxygen flow of 8 l/min. The airway reaction was measured by use of a Godart spiograph. We assumed that threshold reaction to polymyxin B was obtained when FEV₁ decreased more than 15% within 7 or 15 min of the inhalation. Three puffs were performed each time, and the highest value used in the calculation. Details of the method have been described earlier (4).

Preparation of dilutions

Polymyxin B sulphate was diluted in an aqueous Ca^{++} and Mg^{++} at a concentration between 10^{-3} and 10^{-6} $\mu\text{g/ml}$ and distributed in wells of microtitre Cooke plates. Two control wells were used: the first without polymyxin B, the second was a plate with polymyxin B solutions but without calcium or magnesium ions.

Basophil count

The study was performed single blind, the person counting was blind to the blood samples examined. A double-chambered Fuchs-Rosenthal haemocytometer with a volume of 3.2 mm^3 was used, and metachromatic staining with 0.025% toluidine blue in 30% ethyl alcohol adjusted to pH 3.4–3.6 with 4 $\mu\text{l/ml}$ glacial acid, revealed the basophils as the only cells with stained cytoplasm. They showed pink on a clear background. To calculate the number of cells per 1 mm^3 an average count from both chambers was taken and multiplied by 3.13. The subjects with a basophil count below 20 mm^3 were excluded from the study. Degranulation was significant if differences between the study and control wells were above 40% with a count of 30 or 40 basophils in the control wells, and 30% with 60 basophils; according to the principle of Benveniste (1). Preparation of basophils by centrifuging, and washing in Tyrode's solution was performed according to this author. Each subject was studied twice. When the second examination, performed after 2–3 days, differed more than 30% from the first the subject was excluded.

Statistical analysis. The distribution did not seem to be gaussian. We used the median test (the significance level 0.05).

RESULTS

Inhalation test

Fifteen or 26 atopic asthmatic patients had significant bronchial obstruction after inhalation of polymyxin B. Some patients who did

not respond to polymyxin B had slight dyspnea after the provocation but the fall in FEV_1 was slight.

Basophil degranulation

There was no significant difference in total basophil count between the group of atopics and healthy subjects.

No significant degranulation was found in the group of healthy controls, while 17 of 26 asthmatic patients showed basophil degranulation *in vitro*. The basophil reaction was statistically significant for all concentrations in the group of atopics. As a rule the inhalation test was positive in those patients in whom basophil degranulation *in vitro* had been previously observed.

DISCUSSION

Polymyxin B is a histamine-releasing agent whose action is known from animal experi-

Table 1

Basophil reaction to polymyxin B in healthy subjects and asthmatic patients

Concentrations of polymyxin B in $\mu\text{g/ml}$	Number of basophils $\pm$ SD in 1 mm^3	
	Healthy subjects	Asthmatic patients
Control	198 ± 91	367 ± 205
10^{-3}	173 ± 90	260 ± 175
10^{-4}	176 ± 82	258 ± 180
10^{-5}	179 ± 90	197 ± 181
10^{-6}	178 ± 87	336 ± 189

Table 2

Relationship between results of inhalation test and basophil degranulation *in vitro* in 26 asthmatic patients

	Degranulation test <i>in vitro</i>	
	+	-
Inhalation test		
+	14	1
-	3	8

ments (6). The inhalation test is most frequently positive in atopic asthma and is recommended for detecting the allergic background of bronchial obstruction (4). Some authors suggest that increased reactivity in asthma is not limited to the bronchi but also exists in the mediator releasing system (5, 8). There was a significant correlation between bronchial reactivity to histamine and spontaneous histamine release in asthmatic patients (8). Human basophils are more susceptible to D₂O in allergic than in healthy subjects (3, 10), thus, it might be assumed that inhaled polymyxin B produces airway obstruction via degranulation of the sensitized mast cells in asthmatic patients. We noted significant basophil degranulation in allergic persons. Some authors suggest that histamine liberators do not degranulate basophils of healthy humans (9). We did not observe any degranulation in healthy controls either.

Findley suggested that basophils exhibit "releasability" to D₂O in allergic subjects (3). Our results point to the existence of such "releasability" also to polymyxin B. The close relationship between basophil "releasability" and bronchial hyperreactivity to inhaled polymyxin B suggests that the nature of both phenomena is similar, and bronchospasm after polymyxin B is due to mast cell degranulation. It is of interest that not all asthmatics react to inhaled or *in vitro* polymyxin B. It is possible that negative inhalation tests may be due to penetration of the polypeptide to mast cells, although it seems more probable that there are differences in "releasability" of basophils and mast cells among atopic asthmatic patients.

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