

Pseudomonas Aeruginosa Allergy in Cystic Fibrosis

Involvement of Histamine Release in the Pathogenesis of Lung Tissue Damage

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Basophil histamine release by *P. aeruginosa* standard antigen was examined in cystic fibrosis patients chronically infected with mucoid *P. aeruginosa* (CF + P) and with pronounced antibody response against these bacteria, and in patients without *P. aeruginosa* infection (CF – P). All the patients showed eosinophil counts and total IgE, which did not differ significantly from that of normal persons. In the absence of patient's sera, histamine release was only found in two patients in the CF + P group, indicating that type I allergy to *P. aeruginosa* is not predominating in cystic fibrosis.

In the presence of patients' sera significantly more of the CF + P patients responded to *P. aeruginosa* with histamine release compared with the CF – P patients. The response was lost by complement inactivation and regained by reconstitution of the complement activity. The involvement of a type III-mediated complement-dependent histamine release is therefore suggested in the pathogenesis of lung damage in cystic fibrosis.

Key words: allergic type I reaction; allergic type III reaction; complement; cystic fibrosis; histamine release; *Pseudomonas aeruginosa*

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The occurrence of respiratory allergy in cystic fibrosis (CF) patients has been an issue of discussion both with regard to the frequency and with regard to the responsible antigens (9, 11, 19–21). CF patients suffer from chronic and recurrent bacterial lung infection and especially chronic *Pseudomonas aeruginosa* lung infection is frequent (5). In many cases of respiratory allergy in CF these bacteria could therefore be suspected as the causative antigens. In fact, by intracutaneous testing with *P. aeruginosa* cells a typical weal and flare reaction has been found in CF patients chronically infected with *P. aeruginosa*, whereas such responses were not observed in normal

individuals, or in CF patients without *P. aeruginosa* infection (7). Furthermore, immune complex formation between *P. aeruginosa* antigens and specific precipitins formed to these antigens could be responsible for type III reaction in the lungs, since *in vivo* formation of soluble immune complexes has been found in serum and sputum of CF patients and the immune complex activity was more frequent in patients with chronic *P. aeruginosa* lung infection than in patients without this type of infection (12, 13). It therefore seems possible that *P. aeruginosa*-induced type I and type III reactions could be involved in the pathogenesis of lung damage in CF. In the present study

these possibilities were investigated in CF patients by means of the basophil histamine release technique.

PATIENTS

Twenty-four CF patients were included in the study. All patients had a typical history of CF and markedly elevated sweat electrolytes in repeated tests (2).

1) One group consisted of 12 CF patients (seven males, five females, median age 11.5 years, range 6–24) suffering from chronic infection with mucoid strains of *P. aeruginosa* (in the following denoted CF+P) and exhibiting more than 10 different precipitins in serum against water soluble antigens from these bacteria. The patients have been followed as previously described (5, 8). Mean duration of the *P. aeruginosa* respiratory tract infection was 3.6 years (range 1–8). Routine studies of the lung function showed a reduced vital capacity and peak expiratory flow rate as compared with normal values (18). On the average, the vital capacity of the lungs was 1.2 s.d. below mean normal values, and the peak expiratory flow rate was 0.3 s.d. below normal values in healthy persons of the same height.

2) Twelve CF patients (seven males, five females, median age 11.0 years, range 7–18) without *P. aeruginosa* infection (in the following denoted CF–P) and without increased number of serum precipitins (≤ 1 precipitin) (6) against *P. aeruginosa* antigens were also examined. The average vital capacity of their lungs was 0.4 s.d. below mean normal values, and the peak expiratory flow rate was 0.8 s.d. above mean normal values (18). In 10 of the CF–P patients *S. aureus*, *H. influenza*, *E. coli*, or *K. ozaenea* were isolated from the respiratory tract during this study.

Eleven normal persons were included in the study as controls (four males, seven females, median age 10.5, range 6–30). None of the controls had precipitins in serum against *P. aeruginosa*.

METHODS

Cell suspension

Blood samples of 8.5 ml were collected by venipuncture in plastic tubes containing 0.5 ml 0.2 M EDTA (pH 7.0) and 22.5 ml 0.9% NaCl was added to each sample. Monocytes containing approx. 2% basophils were isolated on a Ficoll-Hypaque gradient and finally suspended in Tris-AMC (16). The cell suspension contained 10,000 to 40,000 basophils per ml.

Basophil histamine release

Basophil histamine release was determined in the presence or absence of patient serum. If not otherwise stated, cells and serum were obtained from the patient. In the absence of serum, 200 μ l cell suspensions were incubated in plastic tubes at 37°C for 40 min with 20 μ l *P. aeruginosa* standard antigen at different concentrations. The other experiments were performed with patient serum or normal serum (AB Rhesus negative) in final concentrations of 10%, if not otherwise stated. Samples of 20 μ l serum were incubated at 37°C for 10 min with a mixture of 20 μ l antigen and 60 μ l Tris-AMC. 100 μ l cell suspension was then added and the mixture was incubated at 37°C for 40 min. In both types of experiments the incubation was terminated by adding 3 ml ice-cold Tris-AMC to the samples, which were then centrifuged at 2,000 g for 10 min at 4°C. The residual histamine was determined in the cell sediment by the spectrofluorometric histamine assay (17), and the release of histamine was expressed as a percentage of the total histamine content of the sample. Correction was made for the spontaneous histamine release amounting to 1–5%.

Cell count and histamine content

Basophils were stained and counted and the content of histamine per basophil was determined in the cell suspension as earlier described (10). The total number of leukocytes and eosinophils was counted by routine methods.

P. aeruginosa antibody and standard antigen

The occurrence of circulating precipitating antibodies in serum against *P. aeruginosa* was investigated by means of crossed immunoelectrophoresis (microtechnique) as previously described (6). The antibody response against *P. aeruginosa* was quantitated by counting the number of visible immunoprecipitates on the immunoplates (= number of precipitins) between the *P. aeruginosa* antigen and patient's serum (6). Also the production of polyvalent *P. aeruginosa* standard antigen as a water soluble extract obtained by sonication of whole bacterial cells has previously been described (8).

Total IgE

The total concentration of IgE in serum was determined by the paper radioimmunosorbent technique (PRIST) using Phadebas IgE kits from Pharmacia (Sweden). The concentrations were expressed in U according to WHO standard serum (1 U = 2.4 ng IgE). Normal values amount to: IgE 3–263 U/ml.

Statistical methods

The Mann-Whitney test and Fisher's exact test (fourfold table test, one-tailed) were used and a significance level of 5% was chosen (1).

RESULTS

Histamine release in the presence of serum

Serum concentration. Basophil histamine release by *P. aeruginosa* standard antigen was determined in the presence of serum from a CF+P patient. The optimal concentration of serum for basophil histamine release is seen from Table 1. A final concentration of 10% serum was found to yield maximal release of histamine. When patients' serum was replaced by normal serum, no histamine release was obtained.

CF+P patients. In the presence of the patients' own serum, the *P. aeruginosa* response was

Table 1

Basophil histamine release in a CF+P patient by *P. aeruginosa* in the presence of patient's serum

P.A.*	% Histamine release				
	Patient serum				
	0	0.1%	1.0%	10%	20%
10 ⁻⁵	0	2	1	0	0
10 ⁻⁴	0	2	2	7	0
10 ⁻³	0	5	0	18	0
10 ⁻²	0	0	0	21	0

Note: No histamine release was obtained when patient's serum was replaced by normal serum.

**P. aeruginosa* standard antigen, final protein concentration mg/ml.

examined in all CF+P patients. Table 2 shows that half of the 12 CF+P patients responded to *P. aeruginosa* standard antigen in a final concentration of 10⁻² mg/ml, whereas only 33% of the patients responded to concentrations of 10⁻³ and 10⁻⁴ mg/ml. The number of *P. aeruginosa* precipitins ranged from 11 to 46, and no difference was found between the groups of responders and non-responders. In order to investigate the possibility of the *P. aeruginosa* response being due to a complement-mediated release of histamine by *P. aeruginosa* antigen-antibody complexes a single responder of the CF+P group was examined. In the presence of serum from the patient, *P. aeruginosa* antigens caused a histamine release between 21 and 25%, whereas no release was observed in the absence of serum. By heating the serum to 56°C for 30 min, complement was selectively inactivated. When fresh serum was replaced by heated serum, no histamine release was obtained, but the release was regained by reconstitution of the complement activity by addition of 10% normal serum. Normal serum alone or the combination of normal serum and *P. aeruginosa* standard antigen caused no release of histamine.

CF-P patients. Basophil histamine release by *P. aeruginosa* was determined in CF-P patients in the presence of the patients' own serum. In the CF-P group consisting of 12 patients only

Table 2

Basophil histamine release by *P. aeruginosa* in the presence of serum from CF patients and controls

		% Histamine release			
		<i>P. aeruginosa</i> concentration*			
		Patient No.	10 ⁻²	10 ⁻³	10 ⁻⁴
CF + P patients	1	28	28	16	
	2	17	32	28	
	3	21	18	14	
	4	11	11	10	
	5	11	0	0	
	6	11	0	0	
	7-12	0	0	0	
CF - P patients	1	22	0	0	
	2	14	0	0	
	3	12	0	0	
	4-12	0	0	0	
Normal persons	1	20	0	0	
	2	20	0	0	
	3-11	0	0	0	
CF + P/CF - P:		$P > 0.05$; $P \leq 0.05$; $P \leq 0.05$			
CF - P/controls:		$P > 0.05$; $P > 0.05$; $P > 0.05$			
		(Fisher's test)			

*Final protein concentration mg/ml.

three responded to the *P. aeruginosa* antigen in a final concentration of 10⁻² mg/ml, whereas no response was obtained at concentrations of 10⁻³ and 10⁻⁴ mg/ml, and these results are significantly different from the results obtained with the CF + P group (Table 2).

The possibility of a complement-mediated release of histamine by *P. aeruginosa* antigen was examined in a single responder of the CF - P group. In the presence of serum from the patient, *P. aeruginosa* induced a 14% histamine release, whereas no release was obtained in the absence of serum. The histamine response was lost when the serum was complement-inactivated, but the response was not regained by reconstitution of normal complement activity by addition of 10% normal serum. Similar results were obtained when cells from the patient were replaced by cells from a normal person. In order to further investigate whether the histamine response is

Table 3

Basophil histamine release by *P. aeruginosa* after transfer of serum or cells from a responder¹ (R) to a non-responder² (N-R) and vice versa

		% Histamine release					
Serum Cells		R		N-R		R + N-R ³	
		N-R	R	N-R	R	N-R	R
P.A. ⁴							
10 ⁻³		11	4	0	0	11	10
10 ⁻²		11	21	0	0	15	19

¹CF - P and ²CF + P.

³Combination of sera from responders and non-responders.

⁴*P. aeruginosa* standard antigen, final protein concentration mg/ml.

associated to the serum and not to the cells, transfer experiments were performed (Table 3). When serum from a responder was transferred to cells from a non-responder of the CF + P group, histamine release was obtained, as was the case with the responder's own cells, and no response was obtained by transfer of cells from a responder to serum from a non-responder.

Normal persons. The *P. aeruginosa* response was also examined in 11 normal persons in the presence of their own sera. Two of the 11 individuals responded to *P. aeruginosa* antigens in a final concentration of 10⁻² mg/ml, whereas no response was found in lower concentrations, and these results did not differ from those of the CF - P group (Table 2).

Histamine release in the absence of serum

Basophil histamine release by *P. aeruginosa* standard antigen was determined in the absence of serum in both CF + P and CF - P patients as well as in normal persons. No histamine release was found in normal persons and in CF - P patients, whereas a release was obtained in two patients of the CF + P group who did not respond in the presence of serum. Maximal release of histamine was found with 10⁻³ mg/ml of *P. aeruginosa* standard antigen (Fig. 1).

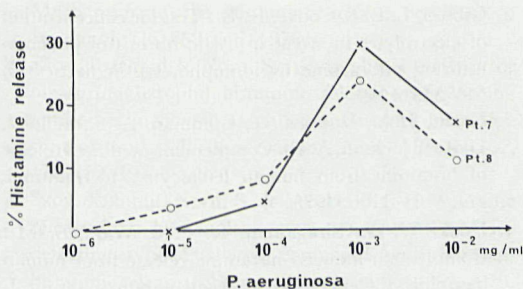


Fig. 1. Basophil histamine release in CF + P patients by *P. aeruginosa* in the absence of serum. Patient numbers refer to Table 2.

Cell count and histamine content

The median value of basophil count in peripheral blood was found to be 55 (range 30–97) per μ l in 11 normal persons. In the groups of CF + P and CF – P patients the median values were 51 (14–100) and 57 (17–77), respectively. The histamine content expressed as pg histamine base per basophil was calculated to 1.27 (0.33–2.80) in control, 1.03 (0.36–2.50) in CF + P patients, and 0.74 (0.48–1.18) in the CF – P group. These values do not differ significantly from one another. A comparison between responders and non-responders within the CF + P and CF – P groups did not show any significant difference with regard to cell count and histamine content.

The median values of eosinophil count per μ l peripheral blood were 300 (90–810) and 220 (60–390) in the CF + P and CF – P groups, respectively. These values did not differ significantly from each other or from the control group of 260 (90–360). Only four patients, all from the CF + P group, showed slightly increased values of eosinophils, and two of these patients showed histamine release by *P. aeruginosa* in the absence of serum.

No significant difference was found in the leukocyte counts, which amounted to 7,200, 7,200 and 6,100 per μ l peripheral blood in CF + P, CF – P, and controls, respectively.

Total IgE

The total concentration of IgE in serum was found to be normal in all CF patients with the

exception of one in the CF + P group and one in the CF – P group, who showed a tendency to higher values, namely 282 and 494 U/ml. These two patients did not release histamine either in the presence or in the absence of serum.

DISCUSSION

In the present study the possibility of *P. aeruginosa*-induced histamine release by type I and type III reactions has been investigated in CF patients by the basophil histamine release technique (16, 17). In the type I reaction antigen reacts with IgE (or IgG) antibody bound to mast cells and basophils, and this interaction, which leads to release of histamine, does not require the presence of serum. In the presence of serum, type III reactions can be induced by activation of serum complement by antigen-antibody complexes via the classical pathway, whereby the split products C3a and C5a are generated, and these split products cause release of histamine from mast cells and basophils (3, 4, 15).

Basophil histamine release by *P. aeruginosa* standard antigen was determined in the absence of serum in 12 CF + P and 12 CF – P patients and was only found in two patients in the CF + P group. This is in agreement with eosinophil counts and total IgE values, which did not differ significantly from that of normal persons. The results therefore show that type I allergy to *P. aeruginosa* is not predominating in CF. On the other hand, we have previously found (7) that all our CF + P patients showed an immediate type weal and flare reaction, which appeared within the first 10 min after *P. aeruginosa* injection (7). However, as also pointed out in the previous paper, these reactions could be due to complement-mediated release of histamine rather than to type I allergy. This explanation is in accordance with the results obtained, when basophils from 50% of the CF + P patients were challenged with *P. aeruginosa* standard antigen in the presence of patients' sera. The possibility of complement-mediated release of histamine

by *P. aeruginosa* antigen-antibody complexes was further supported by the results obtained with serum and basophils from one CF+P patient. When serum from the patient was complement-inactivated, the histamine release was lost, but the release was regained by reconstitution of the complement activity by addition of normal serum, in contrast to the results obtained with a CF-P patient. The results obtained with high concentrations of *P. aeruginosa* antigens in both a few CF-P patients and a few normal persons (Table 2) could possibly be explained by activation of the alternative complement pathway by *P. aeruginosa* lipopolysaccharide.

According to the present results histamine release could be induced in CF + P patients by *P. aeruginosa* immune complex-mediated classical complement activation. This is also supported by our previous findings of *in vivo* formation of soluble immune complexes in serum and sputum sol phase from CF patients, where the immune complex activity was more frequent in CF + P than in CF-P patients (12, 13). Further examination of the sputum sol phase from CF patients showed local complement activation in the lungs, notably in patients suffering from chronic *P. aeruginosa* lung infection (14). We therefore suggest the involvement of a type III-mediated, complement-dependent histamine release in the pathogenesis of lung damage in cystic fibrosis, which could be responsible for the allergic-inflammatory reactions.

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REFERENCES

1. Diem, K. & Lentner, C. (eds.): Documenta Geigy. Scientific tables, 7th edn., pp. 109-123. J. R. Geigy, Basel, 1970.
2. Gibson, L. E. & Cooke, R. E.: Test for concentration of electrolytes in sweat in cystic fibrosis of pancreas utilizing pilocarpine by iontophoresis. *Pediatrics* 23, 545-549, 1959.
3. Grant, J. A., Dupress, E., Goldman, A. S., Schultz, D. R. & Jackson, A. L.: Complement-mediated release of histamine from human leukocytes. *J. Immunol.* 114, 1101-1106, 1975.
4. Hook, W. A., Siraganian, R. P. & Wahl, S. M.: Complement-induced histamine release from human basophils. I. Generation of activity in human serum. *J. Immunol.* 114, 1185-1190, 1975.
5. Høiby, N.: Epidemiological investigations of the respiratory tract bacteriology in patients with cystic fibrosis. *Acta Path. Microbiol. Scand. Sect. B* 82, 541-550, 1974.
6. Høiby, N.: *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. *Acta Path. Microbiol. Scand. Sect. C, Suppl.* 262, 3-96, 1977.
7. Høiby, N., Andersen, V. & Bendixen, G.: *Pseudomonas aeruginosa* infection in cystic fibrosis. *Acta Path. Microbiol. Scand. Sect. C* 83, 459-468, 1975.
8. Høiby, N. & Axelsen, N. H.: Identification and quantitation of precipitins against *Pseudomonas aeruginosa* in patients with cystic fibrosis by means of crossed immunoelectrophoresis with intermediate gel. *Acta Path. Microbiol. Scand. Sect. B* 81, 298-308, 1973.
9. Kulczycki, L. L., Mueller, H. & Shwachman, H.: Respiratory allergy in patients with cystic fibrosis. *J. Am. Med. Assoc.* 175, 358-364, 1961.
10. Permin, H., Skov, P., Stahl, Norn, S. & Juhl, F.: Basophil histamine release by RNA, DNA and aggregated IgG examined in rheumatoid arthritis and systemic lupus erythematosus. *Allergy* 33, 15-23, 1978.
11. Rachelefsky, C. S., Osher, A., Dooley, R. E., Ank, Bonnie & Stiehm, E. R.: Coexistent respiratory allergy in cystic fibrosis. *Am. J. Dis. Child.* 128, 355-359, 1974.
12. Schiøtz, P. O., Høiby, N., Juhl, F., Permin, H., Nielsen, H. & Svehag, S.-E.: Immune complexes in cystic fibrosis. *Acta Path. Microbiol. Scand. Sect. C* 85, 57-64, 1977.
13. Schiøtz, P. O., Nielsen, H., Høiby, N., Glikmann, G. & Svehag, S.-E.: Immune complexes in the sputum of patients with cystic fibrosis suffering from chronic *Pseudomonas aeruginosa* lung infection. *Acta Path. Microbiol. Scand. Sect. C* 86, 37-40, 1978.
14. Schiøtz, P. O., Sørensen, H. & Høiby, N.: Activated complement in the sputum from patients with cystic fibrosis. *Acta Path. Microbiol. Scand. Sect. C* 87, 1-5, 1979.
15. Siraganian, R. P. & Hook, W. A.: Complement-induced histamine release from human basophils. II.

- Mechanism of the histamine release reaction. *J. Immunol.* *116*, 639–646, 1976.
16. Skov, P., Stahl & Norn, S.: A simplified method for measuring basophil histamine release and blocking antibodies in hay fever patients. Basophil histamine content and cell preservation. *Acta Allergol.* *32*, 170–182, 1977.
17. Skov, P., Stahl, Norn, S. & Weeke, B.: ^3H -histamine release from human leukocytes—A new method for detecting type I allergy compared with basophil histamine release technique. *Allergy* *34*, 261–263, 1979.
18. Thuesen Pedersen, J., Lykkegård, E., Faero, O. & Jacobsen, L.: Lung function in children and young adults with cystic fibrosis. *Dan. Med. Bull.* *21*, 12–19, 1974.
19. Van Metre, T. E., Cooke, R. E., Gibson, L. E. & Winkenwerder, W. L.: Evidence of allergy in patients with cystic fibrosis of the pancreas. *J. Allergy* *31*, 141–150, 1960.
20. Warner, J. O., Norman, A. P. & Soothill, J. F.: Cystic fibrosis heterozygosity in the pathogenesis of allergy. *Lancet* *i*, 990–991, 1976.
21. Warner, J. O., Taylor, B. W., Norman, A. P. & Soothill, J. F.: Association of cystic fibrosis with allergy. *Arch. Dis. Child.* *51*, 507–511, 1976.

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