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PATHOMECHANISM AND CLINICAL SIGNS OF MOULD SENSITIZATION IN THE ASTHMOID BRONCHITIS SYNDROME OF YOUNG CHILDREN

By

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Asthmoid or spasmodic bronchitis is a syndrome which may be caused by several different factors; one of these is bronchial asthma of allergic origin. This syndrome is characterized by rather mild bronchospasms which are frequently recurring, or sometimes continuous; it may or may not be accompanied by fever. The first wheezing attack is most often caused by RS or parainfluenza viral infection (2, 19). In a previous work (13) it was described how, in some cases, moulds in the bronchial tract can be responsible for long-lasting bronchospasms. Prolonged antibiotic and corticoid treatment, or partial immunodeficiency, usually precede this illness, and the symptoms become worse if antibiotics are reintroduced.

The criteria for this diagnosis are: 1. The demonstration of fungi: *Conidia* of *aspergillus*, *penicillium*, or atypical hyphae, yeast-like forms in the bronchial secretion obtained by means of bronchoscopy or direct laryngoscopy. 2. The cultivation of these moulds. 3. The demonstration of an immune reaction against the same agents by means of lymphoblastic transformation. 4. Successful antimycotic therapy (13). The syndrome

of spasmodic bronchitis caused by mould infection and allergy is often accompanied by penicillin hypersensitivity. Nevertheless, the exact immunological mechanism of this fungal infection and allergy in young children has not so far been elucidated because eosinophilia is mostly absent from both the secretion and the blood. Skin tests, if performed, exhibit rather a retarded reaction.

MATERIAL AND METHODS

This work was done during a three-year period in a general pediatric department; the instruments and the personnel were the same in the control as in the asthmatic cases. The secretions from tracheostomized children were examined repeatedly, at intervals of 1-2 weeks.

We examined bronchial secretions obtained by means of bronchoscopy or laryngoscopy from patients with asthmoid bronchitis or bronchial asthma whose symptoms did not ameliorate within two weeks of conventional treatment (spasmolytics, diet, corticosteroids, antibiotics). Secretions were cultivated on Sabouraud media.

Bronchial secretions obtained in the same way from patients with different diseases were similarly cultivated on Sabouraud media, and served as control material.

The patients' sera were examined by means of Ouchterlony double diffusion in gel (1) using mould extracts as antigens, but there was no precipitation line.

The demonstration of non-precipitating, heterocytotrophic IgG-antibodies in the sera was performed by means of Ováry's method (14): passive cutaneous anaphylaxis (PCA) in guinea-pigs. Serial (1:4) dilutions of the patient's serum were injected on the left, and of the control serum on the right side of the dorsal skin. The quantity of antigenic material (Bencard extract), given intravenously with Evans blue, was 0.5 mg. Positive reactions to the patient's serum were found in 4 cases up to a dilution of 1:16, while two doubtful cases proved negative. Each material was tested in at least two animals; the latter often also exhibited signs of generalized anaphylactic shock, and even succumbed to it. Lymphoblastic transformation was performed after the cultivation of the patient's leukocytes in Parker's 199 medium. Labelled thymidine uptakes of samples exposed to the antigenic extract (Bencard, diluted 1:50) were compared and the ratio to the blank control was recorded (21).

Lymphoblastic transformation was carried out as a "blind" examination: the operator worked in another institute and did not know the patient's history. The transformation of lymphoblast cultivated in the presence of mould extract did not occur in healthy control persons, and even non-asthmatic patients with tracheostomy who harboured moulds in the respiratory tract displayed negative results.

TABLE 1

Results of Mould Cultivations from Tracheal and Bronchial Secretions (Not selected).

	Mould positive (except Candida)	Candida		Total
		positive	negative	
Spasmodic bronchitis and bronchial asthma	13 (34%)	7 (19%)	18 (47%)	38
Severe pneumonia, treated with antibiotics.				
Tracheostomies.	17 (19%)	19 (22%)	52 (59%)	88
Premature babies	7 (13%)	12 (23%)	33 (64%)	52
Cong. heart disease	2 (13%)	7 (53%)	6 (34%)	15
Other cases	5 (10%)	9 (18%)	36 (72%)	50
Total non-asthmatic cases	31 (15%)	47 (23%)	127 (62%)	205
	44 (18%)	54 (22%)	145 (60%)	243

Natamycine therapy was performed through a nebulizer. The original suspension (Pimafulcine, Mycofarm, Delft) was a concentrated one: 2.5 per cent; prior to use it was diluted ten- or twentyfold, and was then administered as an inhalation. The daily dose was 2.5-5.0 mg in three parts (7, 15, 16).

RESULTS

Instruments used to obtain bronchial secretions may be contaminated with moulds, and this can be a source of misleading positivity. In order to check on this possibility, all material obtained by bronchoscopy from patients with different diseases was also examined on Sabouraud's agar. Moulds other than *Candida* from bronchial secretions were cultivated in 34 per cent of children suffering from bronchospasms, while the overall rate of positivity was only 18 per cent; in the non-asthmatic group this was 15 per cent (Table 1).

Thus, the frequent cultivation of moulds does not seem to be artificial; the demonstrated difference is significant ($P < 0.01$). The non-asthmatic patients harbouring moulds in the

TABLE
Proved Cases of Allergic Bronchospasms

Name	Age (years)	Sex	Cultivation of fungi from the respiratory tract
J. G.	10-12	m	Penicillium Throat: Penic.
K. E.	6-12	f	Penicillium (smear and growth)
V. É.	3	f	Penicillium
P. Z.	1	m	Aspergillus fum. (smear and growth) Stool: Aspergil.
R. G.	2	f	Penicillium
T. A. Bronchial asthma	4	f	Trichoderma viride.
K. L.	1	m	Penicillium (Tracheostomy)
F. I.	2	f	Aspergillus nidulans (Tracheostomy)
H. R. Bronchitis asthma	9	f	Aspergillus fum. in the throat
G. Zs. Bronchitis asthma	3	m	Aspergillus fumigatus
K. E.	1	f	Aspergillus fumigatus
Sz. T. Bronchitis asthma	12	f	Aspergillus fumigatus
P. J. Bronchial asthma	8	m	Fusarium solani in the throat
G. T. Bronchial asthma	6	m	Penicillium fr. in the throat
G. Á.	3	f	Penicillium nigricans

Associated with Mould Infection.

Immunology M-6: Penicillium M-2: Aspergil. Lybl.: Lymphoblast	Allergy		Therapy
	Penicillin	Other	
M-6: 1.8 (lybl.) Penicillin 2.1 PCS 1/8 skin test ++ (late)	Yes asthma	Wheat Milk	Natamyc. success
	Yes asthma	-	Natamyc. success
M-6: 2.1 (lybl.) Penicillin 2.3	Yes Urtic.	Milk	Amphot. No KJ: Yes
M-2: 2.6 (lybl.) M-6: 1.9 Penicillin 1.8 PCA: 1/8 (M-2)	Yes Urtic.	No	Natamyc. doubtf.
M-6: 2.1 (lybl.) Penicillin 1.0	No	Milk?	Finishing antibiotics
M-6: 2.2 (lybl.) Penicillin 1.4 M-6 skin test: asthmatic attack	Yes Urtic.	Milk Hormodendr.	Natamyc. No KJ: desensit. success
M-6: skin prick test	No		Not tried
M-6: 1.5	No	No	Natamyc. success
M-2: skin test intracut. +++	No	House dust	desensit. success
M-6: 3.0 (lybl.) Penicillin 2.3 PCA: 1/16 (M-2)	Yes asthma	Milk Egg	Natamyc. success
PCA: 1/16 (M-2)	No	Milk	Natamyc. doubtf.
M-2: skin test intracut. ++	No	House dust	KJ: desensit. success
Fusarium extr. skin test i.c. ++ (Dil. 1/1000)	Yes Urtic.	House dust	Flat change success
M-6: skin test i.c. ++	No	Milk Fungi	Flat change
neg. neg. PCA: neg. LE ph. pos.	No	No	Natamyc. KJ: success

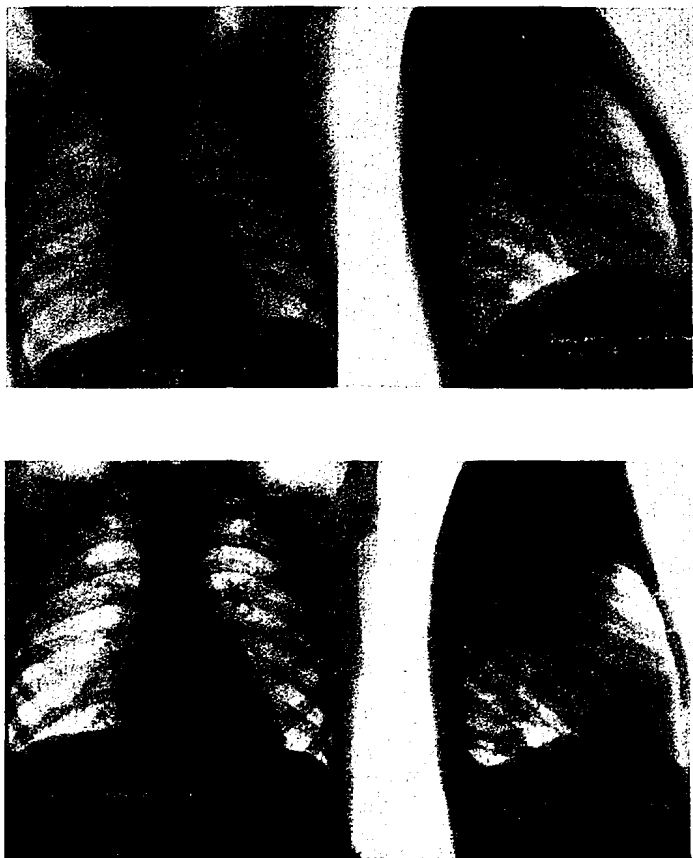


Fig. 1.

X-ray pictures of a patients (V.É., 21 months old) suffering from spasmodic bronchitis caused by moulds.

- a. After antibiotic treatment and dietetic trials, before antimycotics.
- b. After inhalations of Amphotericin-B and long-term (8 months) Potassium Iodide treatment.

respiratory tract suffered from immunodeficiency; some were premature, or newborn babies; others had had severe pneumonia and had received long-lasting antibiotic treatment; some of them had a permanent tracheostomy too.

The frequency of moulds other than *Candida* was 15–20 per cent in each of these disease-groups, i.e. half that for the asthmatics.

However, the fungal aetiology of the bronchospasms was considered proven if both cultivation and immunological examinations established the role of the same mould. These criteria were fulfilled in 15 cases in which the positive findings of the cultivations agreed with the results of lymphoblastic transformation; guinea-pig PCA, respiratory provocation, or skin tests with the extract of the same mould. In all such cases the patients improved with a specific therapy of Natamycine, potassium iodide, or specific hyposensitization in cases of classical bronchial asthma (Table 2). X-ray pictures clearly show the effectiveness of antimycotic treatment (Fig. 1).

In 6 other cases fungal allergy was only suspected, but not proved. Mould was demonstrated in the respiratory tract, but immunological examinations were not performed, or were negative; in some cases positive results were found with other fungal extracts.

There were two emergency cases: both were undergoing their first asthmatic attack and it was necessary to perform tracheotomies; one of them also needed assisted breathing for one day. Both harboured *Aspergillus flavus* in the tracheal secretion, but their later skin reactions were negative to *Aspergillus* extract.

DISCUSSION

Pulmonary aspergillosis in adults is a well-known but rare illness (8, 9, 12). Such an infiltrative pulmonary disease caused by *Aspergillus* in a 9-year-old child was described by Slavin (17). Our observations (13) have shown that *Aspergillus* and *Penicillium* moulds can also cause the asthmoid bronchitis syn-

Name	Sex	Age (years)	Cultivation of fungi	
			Respiratory tract	Throat
L. A.	m	3	Not done	Mucor
P. T.	m	10	Not done	Asperg. fumig i
V. Á.	f	3	Not done	Asperg. fumig i
L. E.	f	2	Not done	-
F. M.	m	2	Aspergillus flavus (Tracheotomy)	
T. J.	m	3	Aspergillus flavus (Tracheotomy)	

drome of infants and young children. Since Slavin's publication in 1971, other authors have described similar cases (3, 10, 11). Our present results show that this fungal aetiology of spasmodic bronchitis is not rare: we have observed 9 new cases in three years. Moulds were isolated from the upper respiratory tract of adult asthmatic patients by Tomsiková & Zavázal (18), while we have also succeeded in cultivating the fungi from the lower respiratory tract of children. Statistical comparison with other material obtained by bronchoscopy has shown that the positivity of asthmatic patients cannot be due to artificial contamination.

Immunologic examinations earlier showed that patients harbouring moulds in the respiratory tract display lymphoblastic transformation with the extracts; we have now established the heterogeneity of the immune reaction: some cases showed immediate skin or bronchial reaction to the test material, whereas others had a delayed reaction. The lack of blood and

ably Associated with Mould Infection.

Immunology	Allergy		Therapy
	Penicillin	Other	
Not done	No	Milk	Natamyc. success
Cladosp. pos. (skin test)	—	—	Desens. success
Stemp. pos. (skin test)	No	No	Flat change success
Lybl. Peni. 2.3 Peni. 20.6	Yes		Finishing penicillin therapy
Lybl. penic. 1.0, Asperg. 1.0			Natamyc. success
Skin tests: neg.	Yes	No	—

secretory eosinophilia and the positivity of the guinea-pig PCA test in four out of five cases examined suggest the role of an Arthus-type reaction.

The favourable results of antimycotic treatment in contrast with the preceding inefficacy of antibiotics support the fungal aetiology. In some mild cases it was sufficient to stop broad-spectrum antibiotic treatment or to move the patient from a damp dwelling to a dry flat. The patients displayed a normal sedimentation rate of red blood cells, and no fever or leucocytosis, which indicates the unimportance of bacterial infection and suggests the fungal aetiology.

In contrast to these allergic asthmoid bronchitis cases, there were two patients in whom *Aspergillus flavus* was isolated from the respiratory tract; both were severe cases endangering the lives of the patients, and it was not possible to establish allergy or immunoreaction to this mould which suggests a direct toxic action. The mould produces aflatoxin,

which is capable of inducing severe damage in animal cells (4, 5). Deicke & Gemeinhardt (6) recently described fatal necrotic bronchiolitis in an adult; our two cured severe asthmatic cases might be of similar pathomechanism. Nonetheless, this flavus type of *Aspergillus* was not isolated from any other asthmatic patient.

The 21 cases described here represent 5 per cent of our patients suffering from bronchial asthma or asthmoid bronchitis during a 3-year period. This shows that this fungal pathogenesis cannot be dismissed as a negligible factor among the causes of chronic bronchospasm in young children.

SUMMARY

21 cases of infantile asthmoid bronchitis or bronchial asthma are described in which moulds could be cultivated from tracheobronchial secretions obtained by bronchoscopy. In 15 of these cases the mould aetiology was also proven by immunological methods: lymphoblastic transformation, and skin tests. 4 out of 5 sera examined gave a positive PCA test with the mould extract in guinea-pigs suggesting an Arthus-type reaction. In two emergency asthmatic cases *Aspergillus flavus* was cultivated from the bronchial secretion obtained after tracheotomy; the lack of immunoreactivity in these cases suggests the aetiological role of aflatoxin. The cases described represent 5 per cent of the asthmoid bronchitis or bronchial asthma patients treated in this pediatric department.

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