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INFLUENZA VIRUS SENSITISATION IN BRONCHIAL ASTHMA

*A study of bronchial provocation tests with
Influenza Virus and Bacterial Extracts*

By

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INTRODUCTION

Bacterial sensitisation occupies a significant place in the aetiology of bronchial asthma in Hungary. Following the investigations of K. Hajos, we prepared in our own laboratory the most frequently encountered strains of bacteria responsible for infections of the upper and lower respiratory tract and for subsequent development of bronchial asthma, from cultures of diseased focal areas and tracheobronchial secretion. Until recently these extracts were employed for routine skin testing. Clinical practice and comparative studies revealed that standard solutions of similar bacterial extracts (Bencard skin and inhalation testing solutions) gave more dependable results.

The most commonly encountered infections responsible for the development of bronchial asthma are common cold, usually complicated with bronchopneumonia, acute bronchitis, whooping-cough and tuberculosis with pleurisy. During the Asiatic influenza pandemic in the fall of 1957 we observed the recurrence of asthmatic attacks in symptom free individuals as well as development of bronchial asthma after the infection subsided. A detailed study of the relationship of influenza and asthma suggested that influenza viruses might be important causes of asthma. We found it necessary, in keeping with clin-

ical experiments, to accumulate definite proof that viral allergy may be of pathogenic significance.

Since 1931, when the first virus strain (A swine) was isolated, the study of virology has occupied a significant place in the spectrum of current medical problems. Two distinctly different antigenic types—A and B virus—with variants replacing previously prevalent strains, were found to cause classic influenza. Laboratory analysis and the failure to achieve protection against the 1947–48 epidemic with the original vaccines made it evident that the A virus, which had been in circulation until 1946, changed and a marked shift occurred to A₁ types. In 1957, A₂/Asian types became prevalent and may yet prevail for several years to come. The change of B strains presents a somewhat different pattern. B Lee, isolated 20 years ago and B₁ GL in 1954, prevailed during the past seven years and was seen in children in 1959. Furthermore, it seems that B Lee is an equivalent of the contemporary type B virus.

Our subsequent investigations are based on the antigen-antibody reactions of influenza viruses. According to Hennisch, the virus gives a specific precipitate pattern with its homologous anti-serum. Suppression of the dominant antigen of a virus to that of a secondary antigen and the new dominant of a secondary antigen, is brought about by an increasing antibody titre in the general population, thereby hiding the propagation of the virus. Arising out of observations of the 1957 pandemic, Takátsy assumed that the extinction of acquired immunity to the A₁ strain is the consequence of a shift in the pattern of the virus and is favoured by previously acquired immunity.

It follows from this theory that antibodies to the Asian strains of 1957 were present in individuals between 40 and 70 years of age. Furthermore, as a result of virus mutation, the similarity of A₂ and B Lee strains is based on the presence of an identical antigen.

As a result of the evidence concerning the antigenic character of the virus, mono and polyvalent influenza vaccines were introduced into basic immunological therapy (Dull, Jensen). However, they are limited in their effectiveness. Their activity

depends on limiting the spread of the virus; thus, propagation of the virus causes influenzal symptoms whilst inactivating methods inhibit the immune reaction. Furthermore, it has to be borne in mind that allergic reactions to egg, allantois and antibiotics may develop in hypersensitive patients.

The untoward effects of influenza on bronchial asthma are based on the observations which follow. It has been recorded that patients suffering from chronic bronchopulmonary diseases had a predisposition to severe influenzal complications in consequence of decreased resistance of the organism. In addition, we noticed development or persistence of attacks in the febrile stage, whereas in other infections, e.g. pneumonia, an amelioration of symptoms occurs when the patients are running high temperatures.

Only meagre reports are available on testing methods with influenza virus extracts. Most of the published data based on skin reactions indicates that allergy to the egg-protein component of the vaccine accounted for the majority of positive results.

The purpose of this paper is to investigate the significance of influenzal sensitisation, using direct bronchial provocation tests for the reproduction of the antigen-antibody reaction in the target tissue of bronchial asthma. Purified viral extracts were prepared in order to avoid non-specific reactions.

MATERIALS AND METHODS

From 262 patients with infective asthma, we assembled two separate groups for investigations with different bacterial and influenza virus extracts:—

Group I—152 asthmatics, with a history of influenza at onset or during the latter course of their basic illness in 118 (77 %). Testing material was as follows:—

1. Bacterial extracts—
 - (a) *H. influenzae*

- (b) F.2, Bencard testing solution containing suspensions of *N. catarrhalis*, *H. influenzae* type A, *Staph. aureus* and *K. pneumoniae*.
- 2. Influenza virus extracts—
 - (a) A.2 Singapore
 - (b) A PR 8
 - (c) B Lee

The *H. influenzae* extract had been prepared in our own laboratory and the influenza virus extracts by the Virology Research Unit of the State Institute of Public Health (Dr. Farks, Dr. Barb and Dr. Takátsy).

For the virus extracts a special procedure had been established to assess the allergenic activity of the virus extract alone. For this reason, penicillin and streptomycin had been omitted and the allantois content reduced to a minimum. We used 0.1 ml of the undiluted virus vaccine, a 10^{-9} solution of the stock vaccine for skin testing and 0.5 ml (two minutes inhalation) for bronchial testing in a 1:2 dilution.

The positive skin reaction was indicated by a 20 mm wheal after 24 hours. A positive provocation test consisted of a bronchial spasm over 20 % in the first 1–15 minutes after inhalation.

Group II—110 patients with influenzal history in 67 (61 %). We used only the bronchial testing methods with following extracts:—

- 1. F.2
- 2. Influenza virus vaccine prepared at the Wright-Fleming Institute of Microbiology, containing a mixture of equal parts of the inactivated viruses A/Singapore 1/57 and B/England 1/59, having a potency by titre of 2500 to 5000/10–20,000 haemagglutinating units per ml obtained by the courtesy of Dr. Milner, Director of the Bencard Allergy Division.

For bronchial testing we used the undiluted solutions, 0.25 ml were inhaled in 90 seconds. Control tests were undertaken with the standard control solution for bacterial inhalants.

The patient's bronchial response was graded positive if the decrease of vital capacity VC exceeded 200 ml, the decrease of the Tiffeneau coefficient (Tiff.) 10 % and the bronchial spasm 10 %. The reactions were recorded 1-15 minutes after inhalation, all reactions were discarded if a similar result was obtained with the control solution.

In order to examine the reactivity of the respiratory tract with other bacterial extracts, we selected a group of 100 asthmatics (Group III) with influenza in 16 (16 %) and no infectious aetiology in 13 (13 %). Inhalation tests were undertaken with the following standard bronchial testing solutions (Bencard) :—

1. F.1 (Strep. pneumoniae, Strep. Lancefield Groups A, C, G, Strep. salivarius)
2. F.2 (see above)
3. F.4 (Strep. viridans)
4. F.5 (B. fusiformis, E. coli, Pseudomonas aeruginosa, Coryne. hofmanni, Staph. albus)

The positive results were graded as above.

The apparatus for bronchial testing consisted of a Krogh-type spiograph and a Dräger nebulizer. Technique and evaluation of the results have been described in detail in a previous paper.

RESULTS

In Group I we compared skin tests and bronchial tests using extracts of bacterial and viral cultures occurring in Hungary. The average age of patients at the time of examination was 40 years. The peak incidence was found to be in the 30-40 year group. Sex distribution was similar for males and females. The results are summarized in Table I.

TABLE I

Skin and bronchial reaction with Influenza Virus and H. influenzae extracts in 152 asthmatics. (History of Influenza in 118 (77 %). (See Group I).

Number of cases	Allergen	Skin test		Bronchial test		History of influenza		Reactions of skin and bronchial test corresponding with history	
		pos.	neg.	pos.	neg.	pos.	neg.	Skin test	Bronchial test
61	H. infl.	38	23	28	33	43	18	28	30
		62%		45%		70%		46%	49%
80	F. 2	22	58	28	52	66	14	30	16
		27%		35%		82%		37%	20%
72	A 2	46	26	22	50	52	20	40	47
	Singapore	63%		30%		72%		55%	65%
80	A PR 8	42	38	32	48	66	14	42	31
		52%		40%		82%		52%	39%
80	B Lee	40	40	30	50	66	14	38	42
		50%		37%		82%		47%	52%

Table I shows that about 40-50 % of skin and bronchial reactions corresponded with the influenzal history, suggesting a similar reliability of both testing methods. However, if the positive and negative results are taken separately, the relationship of negative skin tests, positive bronchial tests and anamnestic data become evident. It should be pointed out that many positive skin reactions are unspecific, whilst the bronchial spasm after viral or bacterial exposure may well indicate specific sensitisation.

In *Group II* influenza and common cold were intimately related to allergic sensitisation in the majority of cases. The anamnestic data are demonstrated in Table II.

Reference to Table II shows that 46 patients (42.5 %) were under 40 years of age, asthma persisted for 6 years or more in 60 (54 %). Sex distribution was identical for males and females. These data indicate that about half of the cases were chronic asthmatics without coexisting non-allergic complications liable to influence the specificity of the reactions.

In 83 patients (75 %) asthma developed after common

TABLE II

*Incidence of anamnestic data in 110 patients with infective bronchial asthma.
(See Group II).*

Duration of Asthma					
Age	Up to 1	2-5	6-10	11-20	21-33
No. of patients	16	34	28	20	12

Age Distribution of Patients

Age	6-10	11-20	21-30	31-40	41-50	51-60	61-70	71-74
No. of patients...	3	12	17	14	23	26	13	2
	14.5%		28%		20%	23%		14.5%

Infections related to onset of asthma

Common cold + pneumonia	33 (30%)
Common cold + influenza	50 (45%)
Influenza	17 (16%)
Other infections (Tbc, Pertussis)	10 (9%)

cold and of these 50 (56 %) deteriorated after influenza. Finally, 17 patients (16 %) had their first attack during the Asiatic influenza pandemic of 1957/58.

Symbols.

	= total number of cases		= history of miscellaneous infections (tbc, pertussis)
	= asthma after influenza		= Br. sp. positive with F ₂ alone
	= asthma after common cold and influenza		= Br. sp. positive with influenza virus vaccine alone
	= asthma after common cold		= Br. sp. positive with F ₂ and influenza virus vaccine
			= Br. sp. negative

Figs. 1 and 2 show the incidence of infective diseases accounting for sensitisation and the number of positive bronchial reactions with F.2 and influenza virus inhalations.

Fig. 1 reveals that the majority of patients attributed their first asthmatic attack to severe common cold. Recurrence of troublesome asthma followed influenzal infections during the subsequent years.

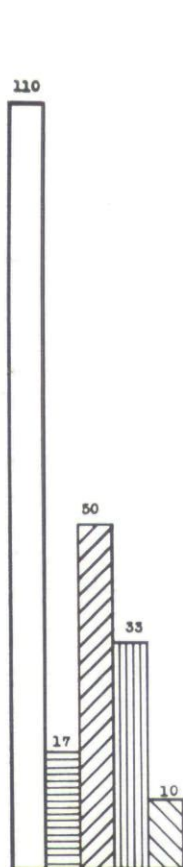


Fig. 1.

Distribution of history in 110 patients with infective asthma.

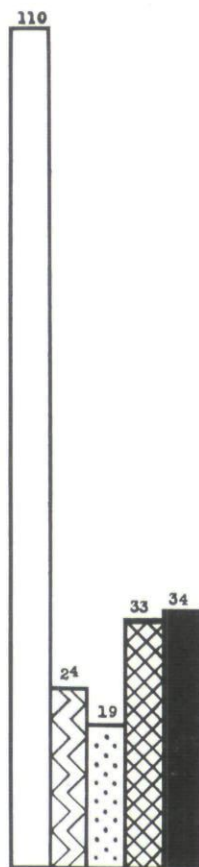


Fig. 2.

Distribution of positive bronchial spasm after bacterial extract and influenza virus inhalation, in 110 patients with infective bronchial asthma.

Fig. 2 shows that both F.2 (*H. influenzae*) and virus inhalations provoked a clear-cut positive bronchial reaction in approximately half of the cases. The correlation with the influenzal history was better when the virus inhalation was used. Nevertheless, provocation tests with *H. influenzae* alone proved to bear a fairly good relationship to the clinical allergic activity of influenza viruses.

Results of different bronchial tests (VC, Tiff. br. sp.) after F.2 and influenza virus inhalation are summarised in Table III.

TABLE III

Results of bronchial tests after F 2 and influenza virus vaccine inhalation corresponding with influenzal history in 110 asthmatics. (See group II).

Number of cases	Allergen	History of influenza		VC ml		Tiff. %		Br. sp. %		Results corresponding with history		
		pos.	neg.	-200 Ø	-10 Ø	10 Ø	10 Ø	VC	Tiff.	Br. sp.		
110	F. 2	67	43	33	77	37	73	57	53	48	51	48
		60%		30%		33%		52%		44%	46%	44%
110	Influenza virus	67	43	46	64	29	81	52	58	57	59	61
		60%		42%		26%		47%		52%	54%	55%

TABLE IV

Correlation between positive bronchial reactions after F 2 and influenza virus inhalation and influenzal infection in 67 asthmatics with clinically verified influenza. (See group II).

Number of cases	History	Bronchial tests					
		F 2			Influenza virus		
		VC ml -200	Tiff. % -10	Br. sp. % 10	VC ml -200	Tiff. % -10	Br. sp. % 10
17	Influenza at onset of asthma	4 25%	6 37%	8 50%	10 59%	7 44%	9 53%
50	Influenza aggravating asthma	13 26%	15 30%	27 54%	21 42%	13 26%	31 62%
		17 25%	21 31%	35 52%	31 46%	20 30%	40 60%

From a comparison of the three different methods for evaluation of bronchial tests, it appears that they correspond exactly with anamnestic data, though the positive bronchospastic results were greater in number and proved to be a more reliable test for diagnosis.

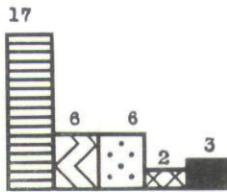


Fig. 3.

Distribution of positive br. sp. after F_2 and influenza virus inhalation in 17 cases of asthma after influenza.

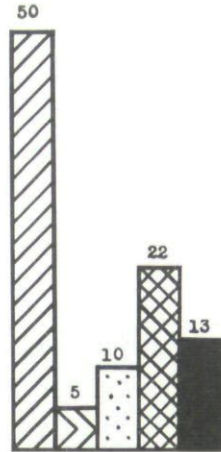


Fig. 4.

Distribution of positive br. sp. after F_2 and influenza virus inhalation in 50 cases of asthma after common cold and influenza.

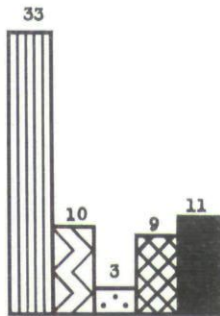


Fig. 5.

Distribution of positive br. sp. after F_2 and influenza virus inhalation in 33 cases of asthma after common cold.

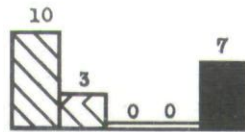


Fig. 6.

Distribution of positive br. sp. after F_2 and influenza virus inhalation in 10 cases of asthma after various infections.

The relationship of anamnesis and bronchial tests in different infective groups is presented in detail in Figs. 3-6 and Table IV.

It is interesting to observe that the bronchospastic effect of F_2 and influenza virus appeared to be identical in the 17 cases

where influenzal infection had been directly responsible for the development of bronchial asthma, whereas in the 50 cases where the first symptoms had been associated with common cold and the aggravation possibly correlated with periods of influenzal incidence, influenza virus reacted more often. The interpretation of multiple reactions is complicated by crossed immunological coverage for additional infectious agents not included among the allergens at hand. Viral sensitivity of the common cold group suggests either a shift of bacterial sensitisation to virus susceptibility, or a synergism of the two allergens

In common cold sensitisation without evidence of influenza the number of cases responding to virus exposure diminished and no reactions to influenza virus were noted in connection with other infections.

The correlation between history and provocation tests would be more significant if the diagnostic uncertainties concerning the definition of common cold, pneumonia, influenza etc. could be eliminated. There was, however, no alternative but to accept and record the anamnestic data as supplied by the patient.

Table V and Figs. 7 and 8 make it evident that the bronchial spasm after bacterial and virus inhalation was accompanied in the majority of cases by a significant decrease of the VC, especially when influenzal sensitisation was present. Furthermore, the incidence of bronchial spasm occurred with greater frequency in those cases in which the infectious history was reliable.

Evaluation of the changes of the Tiffeneau coefficient accorded less information about the causative allergen; it has to be accepted that the Tiffeneau value is fairly constant in a given case of bronchial asthma. Furthermore, the decrease of the VC is generally followed by an approximately equal decrease of the Fev_1 .

In *Group III*, consisting of 100 unselected asthmatics, we employed four groups of bacterial solutions (Bencard F.1, F.2, F.4 and F.5) for bronchial testing in order to estimate general bacterial sensitivity. 87 patients had infective asthma.

TABLE V

Correlation between different bronchial testing methods after F 2 and influenza virus inhalation in 110 cases. (See group II).

Results	VC	Tiff.	Br. sp
Equal	56 51%	37 33%	51 46%
Divergent	54 49%	73 67%	59 54%

In 13 cases asthma developed after diverse non-infective causes (pregnancy, smoke, paint etc.). In this series we wanted to investigate the allergic activity of bacteria involved in infec-

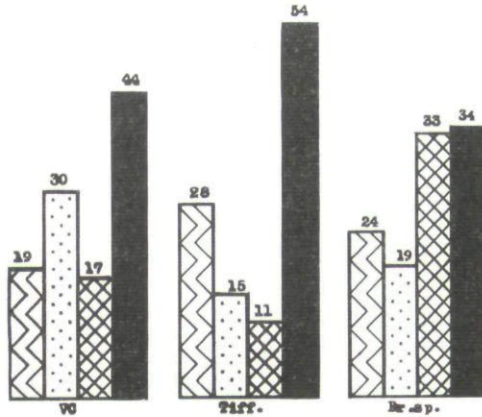


Fig. 7.

Comparison of positive results of different bronchial tests (VC, Tiff., Br. sp.) in 110 patients.

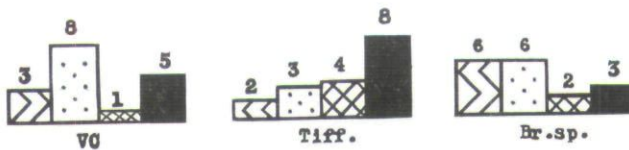


Fig. 8.

Comparison of positive results of different bronchial tests (VC, Tiff., Br. sp.) in 17 patients with asthma after influenza.

tions of the respiratory system for comparison with the results of *H. influenzae* and influenza virus inhalation.

The investigations summarized in Table VI were carried out under the same basic conditions as in Group II (Table III). The results make it evident that asthmatics with an infective history responded in about 80 % of cases to bacteria deriving predominantly from the upper respiratory tract. Streptococcal sensitisation seemed to be prevalent in common cold and influenza. Following other types of infection the susceptibility seemed to be more heterogeneous. After discarding the usual percentage of non-specific reactions, no positive bronchial spasm could be elicited with bacterial inhalation tests in non-infective asthma.

TABLE VI
Results of bronchial tests with bacterial solutions in 100 asthmatics.
(See group III).

Number of cases	History	Bronchial tests (br. sp.)				
		positive				negative
		Str. pneum. saliv. Lancefield A.C.G. (F. 1)	N. catarrh. H. infl. Staph. aur. K. pneum. (F. 2)	Str. virid (F. 4)	Pseudomonas aer. Co. ryne hoim. B. fusiform. B. coli (F. 5)	
41	Common cold	23	14	13	18	5 (13%)
13	Pneumonia	4	3	7	5	1
16	Influenza	8	6	11	7	3 (18%)
17	Various infections (tbc. pertussis, bronchitis, pleurisy)	7	7	2	14	4 (24%)
13	No infection	0	2	2	1	10 (76%)

DISCUSSION AND CONCLUSIONS

In 1933, K. Hajos was the first to correlate influenza with bronchial asthma. He reported that the asthmatic state deteriorated after the acute infectious symptoms. Furthermore, asthma developed in previously healthy individuals. Quarles

van Ufford and Savelberg reported data relating to the effects of Asiatic influenza on asthmatics. Their statistical evaluation indicated an absence of adverse reactions in the majority of these cases. They referred to influenza as a non-specific stress reaction, which lowers the general resistance and increases bacterial sensitisation. Their observations are in agreement with K. Hajos in saying that bronchial asthma per se does not predispose the patient to Asiatic influenza.

The patients reported in the present paper were under our personal supervision and a period of approximately four years was required to accumulate the data.

As a result of the investigation of aetiological factors we found an important relationship between the Asiatic influenza pandemic of 1957/58 or minor epidemics in the fall and early spring of 1959/60 and the development or deterioration of bronchial asthma.

We were surprised to notice the high incidence of patients over the age of 60 years who had been non-allergic prior to being afflicted with influenza. This is doubly significant in view of the fact that genuine asthma was considered to develop more readily under 40 years of age. It seems logical to consider that the prevalent virus strains are a new modification and enhance sensitisation in individuals who had acquired immunity in preceding epidemics. This is supported by the fact that the older age group had been afflicted with influenza either in 1918 or 1933. Our observations seem to support Takátsy's theory concerning the effect of acquired immunity on the shift of virus types. We could even go further and suggest that infections with different virus strains encourage virus sensitisation.

We wish to emphasize that bronchial testing is a fairly reliable proof of aetiologic relationship, since by this means the antigen-antibody reaction is explored in the target-organ. Our previous investigations into the relationship between skin and bronchial testing determined the reliability of the latter method. Gronemeyer and Fuchs reached similar conclusions although they used different allergens and bronchial tests.

Classic influenza of 1957/58 was caused by A₂ (Iksha,

Asiatic, Japan, Singapore), B.1 (GL, England and B Lee) virus strains, with variants of slightly different antigenic type according to geographic distribution. Bronchial tests with influenza virus extracts, prepared both in England and Hungary, revealed the presence of a polyvalent antigen, hence the identical bronchial reactions. Furthermore, when the virus strains were tested separately, the same patients reacted to A.2, B Lee and A + B virus extracts respectively. This observation supports the opinion of Hennisch, who postulated the presence of an identical antigen in the two virus strains.

The presence of antibodies to *H. influenzae* in chronic bronchitis has been discussed by Glynn, who found antibodies in high titre in 66 % of patients with chronic bronchitis and in 21 % of asthmatic patients. According to his report a close association exists between *H. influenzae* and acute infective episodes of the respiratory system without relationship, however, to the duration and severity of the disease or to the presence of active infection. Examining sputum and nasopharyngeal discharge, K. Hajos isolated *H. influenzae* in 24.2 % of a group of 1000 asthmatics. We found that bronchial reactions to *H. influenzae* and influenza virus correlated. These findings may be construed as evidence for an association similar to the relationship of *Proteus* X 19 to *Rickettsia prowazeki* in scrub fever. Our results are such that bronchial testing with *H. influenzae* may indicate influenzal sensitisation.

Our investigations showed that about 50 % of asthmatics afflicted with influenza developed virus sensitivity. The first two virus strains in order of frequency of reactions were A.2 Singapore and B Lee (combined with its contemporary modification B1 GL). Their antigenic role corresponds with their incidence in the development and aggravation of bronchial asthma. This could be demonstrated by obtaining identical bronchial reactions when inhaled separately. The least frequent reactor was A PR 8, prevalent among older patients known to have been afflicted with influenza in 1918 or 1933.

It seems obvious to suggest the introduction of influenza virus desensitisation when influenza virus has been implicated

in bronchial asthma. Nevertheless, the employment of these vaccines involves such problems as technical difficulties of stabilization without risking additional sensitisation with antibiotics and proteins. These problems were taken into consideration during our investigations and led us to exclude all patients with known sensitivity to these agents.

SUMMARY

This paper reports on the effect of Asiatic influenza on a series of 262 patients with infective bronchial asthma. A description is given of the results of provocation tests after *H. influenzae* and influenza virus inhalation. Bronchial tests showed a better correlation with influenzal history than skin testing alone. Reactions to influenza virus were often associated with reactions to *H. influenzae*, which suggested to us that positive bronchial tests with the latter may also indicate viral sensitisation.

An additional group of 100 asthmatics have been tested for sensitivity to bacteria deriving from the upper respiratory tract. We found that only patients with infective asthma responded to direct bronchial testing.

The experiments made it evident that influenzal sensitisation increased after the Asiatic influenza pandemic of 1957/58 as well as after minor epidemics of 1959/60.

It is therefore concluded that specific desensitisation with vaccines plays an important role in the management of asthma in children and young adults.

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