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Secretor Status in Asthma and Hay Fever*

M. A. DENBOROUGH and H. J. DOWNING

From the University of Melbourne Department of Medicine, Royal Melbourne Hospital, Melbourne, Victoria, Australia

The observation that ABO agglutinins are present in a wide variety of pollens (Denborough, 1964) and so must commonly be inhaled, and the knowledge that plant agglutinins are antigenic (Mäkelä, 1957), prompted us to study secretor status in allergic disease of the respiratory tract, namely in asthma and hay fever. The opportunity has been taken to measure the amount of isoagglutinins present in the serum and saliva of these subjects at the same time.

Materials and Methods

Tests were carried out on 435 subjects with asthma and hay fever and 411 healthy control subjects. Some of the subjects with asthma and hay fever were attending Allergy Clinics in Melbourne or the Asthma Foundation of Victoria, some were volunteers from the University of Melbourne, and some were blood donors. Of the 435 patients, 182 had asthma alone, 140 had asthma and hay fever, and 113 had hay fever alone. All the subjects with asthma had unequivocal histories of recurrent airway obstruction without chronic respiratory infection, and those with hay fever gave a history of typical recurrent attacks. Both seasonal and perennial varieties were included. The complaints were seasonal in 53 (29%) of the subjects with asthma alone, in 80 (57%) of those with asthma and hay fever, and in 100 (88·5%) of the subjects with hay fever alone. The 411 controls were all healthy blood donors without a history of asthma or hay fever. The mean age in the group with asthma and hay fever was $41{\cdot}1 \pm 16{\cdot}0$ years and in the controls it was $36{\cdot}3 \pm 12{\cdot}1$ years.

Samples of blood were obtained from all subjects and samples of saliva were collected from 404 of those with asthma and hay fever and from 353 controls. Because there is a seasonal variation in the titre of serum isoagglutinins (Shaw and Stone, 1957), the samples were collected from the subjects with asthma and hay fever and from the controls sequentially. The sample of saliva was collected without artificial stimulation. Half of the sample of saliva was stored immediately at $-15°$ C. until tested for the presence of agglutinins. The other half, for the test of secretor status, was placed in a boiling water bath for 10 minutes before storage at $-15°$ C.

ABO Blood Group. The ABO blood group was assessed by the use of the standard tube method.

Secretor Status. The secretion of ABH antigens in the saliva samples was measured by doubling-dilution agglutination inhibition tests. Samples from a known secretor and non-secretor were tested at the same time. A saline extract of the seed of *Ulex europaeus* was used as a source of anti-H. Each inhibition titration was given a score; complete inhibition counted as 10, $\pm$ as 8, + as 6, ++ as 4, +++ as 2, and ++++ as 0. A subject was regarded as being a non-secretor if his total salivary score was less than 20.

Agglutinins. The amount of isoagglutinins present in serum and saliva was determined using a standard 12-tube doubling-dilution titration. Control samples containing known amounts of isoagglutinins were tested at the same time. The results were read macroscopically and the amount of agglutination present was recorded as ++++, +++, ++, +, or 0. The last tube showing + agglutination was recorded, and each titration was also given a score, each + counting as 1.

The tubes for secretor tests and agglutinin titres were read by a single observer. Individual scores were closely reproducible.

Results

Of the subjects with asthma and hay fever, 196 were men and 239 were women. There were 221 male and 190 female controls. Table I shows that the ABO blood group distribution in the two groups was similar over-all and in both sexes, and differed little from the distribution in a large Australian defence force series (Bryce *et al.*, 1950).

Secretor status was tested in 404 subjects with asthma and hay fever and 353 controls (Table II). There was no significant difference in the incidence of secretors in either sex between the two groups.

Though fewer individuals with asthma and hay fever had isoagglutinins in their saliva than controls, the only statistically significant differences between the groups were in the over-all incidence of anti-A saliva agglutinins in subjects belonging to group O ($\chi^2 = 6{\cdot}0$ for 1 degree of freedom; $p < 0{\cdot}05$),

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TABLE I

ABO BLOOD GROUP DISTRIBUTION IN ASTHMA AND HAY FEVER AND IN CONTROLS

Blood Group	All Subjects				Males				Females				Australian Defence Force (Bryce *et al.*, 1950)	
	Asthma and Hay Fever		Control		Asthma and Hay Fever		Control		Asthma and Hay Fever		Control			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
O	212	48·7	195	47·5	94	48·0	100	45·2	118	49·4	95	50·0	84,034	47·5
A	166	38·2	172	41·8	81	41·3	94	42·5	85	35·6	78	41·1	70,565	39·9
B	48	11·0	33	8·0	18	9·2	21	9·5	30	12·6	12	6·3	17,215	9·7
AB	9	2·1	11	2·7	3	1·5	6	2·7	6	2·5	5	2·6	5,129	2·9
Total	435		411		196		221		239		190		176,943	

TABLE II

SECRETION OF A, B, AND H SUBSTANCES IN SALIVA IN ASTHMA AND HAY FEVER AND IN CONTROLS

Secretion of A, B, and H Substances in Saliva	All Subjects				Males				Females			
	Asthma and Hay Fever		Control		Asthma and Hay Fever		Control		Asthma and Hay Fever		Control	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Secretors	306	75·7	280	79·3	129	74·6	156	79·2	177	76·6	124	79·5
Non-secretors	98	24·3	73	20·7	44	25·4	41	20·8	54	23·4	32	20·5
Total	404		353		173		197		231		156	

and in the incidence of anti-B saliva agglutinins in women of group A ($\chi^2 = 4{\cdot}3$ for 1 degree of freedom; $p < 0{\cdot}05$) (Table III). On the other hand, the mean score of salivary agglutinins did not differ significantly between subjects with asthma and hay fever and controls (Table IV). In both groups the incidence of salivary anti-B agglutinins was greater in O subjects than in those belonging to group A.

Serum agglutinin titres did not differ significantly between subjects with asthma and hay fever and controls (Table V).

No differences were detected in any of these tests between patients with asthma and those with hay fever alone.

Discussion

In studies of secretor status the incidence of non-secretors is usually of the order of 20–25% (Race and Sanger, 1962). This high incidence of non-secretion suggests that the secretor—non-secretor system is an example of human polymorphism according to the definition of Ford (1940), and that selective factors exist for both secretors and non-secretors.

Though it is unlikely to have any effect on natural selection, a clear-cut association between secretor status and disease (duodenal ulcer) has been described (Clarke *et al.*, 1956) and confirmed (McConnell 1966). There may also be an excess of

TABLE III

SALIVA AGGLUTININS IN ASTHMA AND HAY FEVER AND IN CONTROLS

Blood Group	Agglutinins	All Subjects				Males				Females			
		Hay Fever and Asthma		Controls		Hay Fever and Asthma		Controls		Hay Fever and Asthma		Controls	
		No. Tested	Saliva Antibodies Present	No. Tested	Saliva Antibodies Present	No. Tested	Saliva Antibodies Present	No. Tested	Saliva Antibodies Present	No. Tested	Saliva Antibodies Present	No. Tested	Saliva Antibodies Present
O	Anti-A	139	72(51·8%)*	93	64(68·8%)*	62	26(41·9%)	31	20(64·5%)	77	46(59·0%)	62	44(71·0%)
	Anti-B	140	75(53·6%)	93	58(62·4%)	63	24(38·1%)	31	16(51·6%)	77	51(66·2%)	62	42(67·7%)
A	Anti-B	109	25(22·9%)	79	27(34·2%)	52	10(19·2%)	38	7(18·4%)	57	15(26·3%)*	41	20(48·8%)*
B	Anti-A	24	7(29·2%)	15	7(46·7%)	9	3(33·3%)	9	6(66·7%)	15	4(26·7%)	6	1(16·7%)

* $p = < 0{\cdot}05$.

TABLE IV

MEAN SALIVA AGGLUTININ SCORES AND STANDARD ERROR IN ASTHMA AND HAY FEVER AND IN CONTROLS

Blood Group	Agglutinins	All Subjects				Males				Females			
		Hay Fever and Asthma		Controls		Hay Fever and Asthma		Controls		Hay Fever and Asthma		Controls	
		No.	Score	No.	Score	No.	Score	No.	Score	No.	Score	No.	Score
O	Anti-A	139	4·27 ± 0·46	93	4·69 ± 0·49	62	3·31 ± 0·65	31	4·23 ± 0·81	77	5·04 ± 0·64	62	4·92 ± 0·62
	Anti-B	140	4·38 ± 0·55	93	4·75 ± 0·56	63	3·00 ± 0·66	31	2·87 ± 0·73	77	5·51 ± 0·62	62	5·69 ± 0·73
A	Anti-B	109	1·74 ± 0·41	79	1·72 ± 0·39	52	1·75 ± 0·63	38	0·81 ± 0·38	57	1·74 ± 0·63	41	2·59 ± 0·63
B	Anti-A	24	1·00 ± 0·45	15	1·67 ± 0·56	11	0·82 ± 0·55	9	2·56 ± 0·77	13	1·15 ± 0·70	6	0·33 ± 0·33

TABLE V

SERUM AGGLUTININ TITRES IN ASTHMA AND HAY FEVER AND IN CONTROLS

Blood Group	Agglutinins	Subjects Tested	No. Tested	Serum Titre											
				1	2	4	8	16	32	64	128	256	512	1024	2048
O	Anti-A	Asthma and hay fever	166	0	0	0	1 (0·6%)	2 (1·2%)	6 (3·6%)	8 (4·8%)	21 (12·7%)	31 (18·7%)	38 (22·9%)	25 (15·1%)	34 (20·5%)
		Controls	177	0	0	0	2 (1·1%)	11 (6·2%)	16 (9·0%)	18 (10·2%)	19 (10·7%)	31 (17·5%)	33 (18·6%)	24 (13·6%)	23 (13·0%)
	Anti-B	Asthma and hay fever	168	0	0	4 (2·4%)	2 (1·2%)	4 (2·4%)	21 (11·3%)	19 (11·3%)	45 (26·8%)	22 (13·1%)	20 (11·9%)	11 (6·6%)	20 (11·9%)
		Controls	178	0	1 (0·6%)	0	6 (3·4%)	12 (6·7%)	27 (15·2%)	24 (13·5%)	22 (12·4%)	31 (17·4%)	24 (13·5%)	18 (10·1%)	13 (7·3%)
A	Anti-B	Asthma and hay fever	144	0	0	1 (0·7%)	6 (4·2%)	5 (3·5%)	15 (10·5%)	21 (14·6%)	24 (16·7%)	33 (22·9%)	16 (11·1%)	11 (7·6%)	12 (8·3%)
		Controls	154	0	1 (0·6%)	1 (0·6%)	4 (2·6%)	9 (5·8%)	16 (10·4%)	28 (18·2%)	19 (12·3%)	32 (20·8%)	20 (13·0%)	14 (9·1%)	10 (6·0%)
B	Anti-A	Asthma and hay fever	37	0	0	1 (2·7%)	0	0	5 (13·5%)	7 (18·9%)	5 (13·5%)	13 (35·1%)	3 (8·1%)	3 (8·1%)	0
		Controls	31	0	0	0	0	2 (6·4%)	2 (6·4%)	3 (9·6%)	4 (12·8%)	12 (38·7%)	6 (19·4%)	1 (3·2%)	1 (3·2%)

non-secretors among patients with rheumatic fever (Glynn, Glynn, and Holborow 1956, 1959; Clarke, McConnell, and Sheppard, 1960). The reasons for the association between duodenal ulcer and non-secretors are not known. Cain (1957) postulated that it might be related to the ingestion of agglutinins in the seeds of leguminous plants commonly used for food. He suggested that there might be a harmful interaction between these plant agglutinins and cells lining the intestinal tract. This effect would occur in non-secretors but would be neutralized by the blood group substances present in water-soluble form in the intestinal secretions of secretors.

The observation that agglutinins of ABO red cells are found in a wide variety of pollens from grasses, flowers, and trees (Denborough, 1964), and so must commonly be inhaled, raised the possibility that these agglutinins might interact with cells containing blood group antigens in the respiratory epithelium, an effect that would be neutralized in secretors. The knowledge that plant agglutinations may be antigenic (Mäkelä, 1957) prompted us to study secretor status in allergic disease of the respiratory tract. However, in the present study no increase in the incidence of non-secretors in subjects with asthma and hay fever was found.

No significant difference was found either in the amount of isoagglutinins in the saliva or serum of individuals with asthma and hay fever when compared with controls. This part of the study was prompted by the observation that extracts of pollen contain not only ABO agglutinins, but also substances that will inhibit agglutination between ABO red cells and the appropriate isoagglutinins (Denborough, 1964). These agglutination-inhibitors are presumably carbohydrates, chemically similar to those determining the immunological specificity of ABH blood group substances (Watkins and Morgan, 1959), and it is possible that inhalation of such substances in pollen might account for the rise in serum isoagglutinin titres, which occurs in summer (Shaw and Stone, 1957).

The present study confirms previous observations that saliva anti-B isoagglutinins are found more commonly in group O subjects than in those of group A (Prokop, 1961; Jakobowicz, Graydon, and Simmons, 1966; Denborough, Downing, and McCrea, 1967). Salivary isoagglutinins are predominantly IgA immunoglobulins, and there is evidence that

they are transported from serum to saliva by a selective mechanism (Tomasi *et al.*, 1965; South *et al.*, 1966). No difference in this mechanism was detected in the present study between patients with asthma and hay fever and controls.

Summary

A study of the ABO system in asthma and hay fever has shown no significant difference from controls in ABO blood group distribution, secretor status, or the titres of serum and salivary isoagglutinins.

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References

Bryce, L. M., Jakobowicz, R., McArthur, N., and Penrose, L. S. (1950). Blood-group frequencies in a mother and infant sample of the Australian population. *Ann. Eugen. (Lond.)*, **15**, 271.

Cain, A. J. (1957). Possible significance of secretors. *Brit. med. J.*, **1**, 212.

Clarke, C. A., Edwards, J. W., Haddock, D. R. W., Howel-Evans, A. W., McConnell, R. B., and Sheppard, P. M. (1956). ABO blood groups and secretor character in duodenal ulcer. Population and sibship studies. *Brit. med. J.*, **2**, 725.

——, McConnell, R. B., and Sheppard, P. M. (1960). ABO blood groups and secretor character in rheumatic carditis. *ibid.*, **1**, 21.

Denborough, M. A. (1964). Blood groups and disease. *ibid.*, **2**, 190.

——, Downing, H. J., and McCrea, M. G. (1967). **16**, 320. The ABO system in milk and saliva. *Aust. Ann. Med.*, **16**, 320.

Ford, E. B. (1940). Polymorphism and taxonomy. In *The New Systematics*, p. 493. Ed. by Julian Huxley. Clarendon Press, Oxford.

Glynn, A. A., Glynn, L. E., and Holborow, E. J. (1956). The secretor status of rheumatic-fever patients. *Lancet*, **2**, 759.

——, ——, and —— (1959). Secretion of blood-group substances in rheumatic fever. A genetic requirement for susceptibility? *Brit. med. J.*, **2**, 266.

Jakobowicz, R., Graydon, J. J., and Simmons, R. T. (1966). Observations on saliva agglutinins. *Med. J. Aust.*, **1**, 399.

McConnell, R. B. (1966). *The Genetics of Gastro-Intestinal Disorders*, p. 94. Oxford University Press, London.

Mäkelä, O. (1957). Studies in hemagglutinins of leguminosae seeds. *Ann. Med. exp. Fenn.*, Suppl. 11, 17.

Prokop, O. (1961). Bermerkenswertes Überwiegen der Anti-B Ausscheidung in Speichel von-Gegenüber Al-Trägern. *Blut*, **7**, 143.

Race, R. R., and Sanger, R. (1962). *Blood Groups in Man*, 4th ed., p. 228. Blackwell Scientific Publications, Oxford.

Shaw, D. H., and Stone, W. H. (1957). Seasonal variation of naturally occurring isoantibodies of man. Trans. VI Cong. Europ. Soc. Hemat., 724.

South, M. A., Cooper, M. D., Wollheim, F. A., Hong, R., and Good, R. A. (1966). The IgA system. 1. Studies of the transport and immunochemistry of IgA in the saliva. *J. exp. Med.*, **123**, 615.

Tomasi, T. B., Tan, E. M., Solomon, A., and Prendergast, R. A. (1965). Characteristics of an immune system common to certain external secretions. *ibid.*, **121**, 101.

Watkins, W. M., and Morgan, W. T. J. (1959). Possible genetic pathways for the biosynthesis of blood group mucopolysaccharides. *Vox Sang. (Basel)*, **4**, 97.