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A COMPARISON OF THE IMMUNOGLOBULINS IgA, IgG AND IgE IN NASAL SECRETIONS FROM NORMAL AND ASTHMATIC CHILDREN

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SUMMARY

The secreted proteins obtained from nasal washings from eighty-five normal children and fifty asthmatic children, between 6 and 14 years, were investigated by immunological assay for their content of albumin, secretory IgA and IgG. A smaller number of specimens from asthmatic and normal children were examined for their IgE content. There was no statistically significant difference in the levels of albumin, secretory IgA and IgG in the asthmatic and the normal children. The IgE content of nasal wash protein in normal children averaged approximately 70 ng/mg of protein, whereas the IgE content in the asthmatic children averaged greater than 400 ng/mg of wash protein.

INTRODUCTION

The investigation of asthma has defined two broad areas of abnormality. There is an abnormal immunological response, in the extrinsic variety of asthma particularly, and there is an abnormal response to pharmacological agents (Townley *et al.*, 1967). The immunological abnormality is linked to the immediate hypersensitivity response, which is itself dependent on the presence of IgE, and the balance between IgE and blocking antibody. Blocking antibody may be either of IgG or IgA origin (Lichtenstein *et al.*, 1968). In asthmatics, investigation of the serum levels of immunoglobulins has demonstrated inconsistent changes in IgG or IgA as compared to normal individuals. The level of IgE in the serum of asthmatic individuals is increased in a proportion of these individuals, as it is also in individuals with atopic dermatitis and hay fever (Berg & Johansson, 1969). For asthmatics the area that determines attacks of bronchospasm is the mucosa of the respiratory tract. The concentration of pharmacologically active agents and the local tissue susceptibility to these pharmacological agents, determines the degree of bronchospasm, the degree of tissue oedema

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and the rate of mucous secretion. The release of these pharmacologically active substances is determined predominantly by the immediate hypersensitivity response.

Investigation of the levels of immunoglobulins on the mucosa of the respiratory tract is therefore appropriate in evaluating this abnormal immunological response.

Patients and Controls

Asthmatic children, between 6 and 14 years, were selected from those attending an asthma clinic, who had had repeated attacks and whose disease pattern had been observed over more than 6 months. No asthmatic child was on corticosteroid medication, had received hyposensitization therapy or had evidence of upper respiratory infection at the time of study.

Control children were selected from those of 6 to 14 years who were attending the hospital for minor maladies or who had been hospitalized and were convalescing from orthopaedic or surgical conditions. No child studied had clinical evidence of respiratory disease.

MATERIALS AND METHODS

Antisera against IgA, IgG and albumin, and standard normal human sera were purchased from Behringwerke.* Secretory IgA was prepared from normal pooled human colostrum by ion exchange chromatography on diethyl aminoethyl (DEAE) cellulose followed by gel filtration on a column of Sephadex G-200 (Newcomb *et al.*, 1968). Anti-IgE was kindly donated by D. S. Rowe, Lot No. 8930, and IgE concentrations were determined by comparison with WHO normal human sera 67/97. An IgE rich preparation was prepared from an atopic patient's serum by the procedure of Ishizaka & Ishizaka (1967). This preparation was used as a reference sample for the estimation of IgE in some samples of nasal secretions.

Anti-sheep γ-globulin was kindly donated by Dr S. Hogarth-Scott (Melbourne) and Dr D. MacKenzie (Perth).

Collection of specimens was effected by using a lavage technique tolerable to a 6-year-old child. Essentially the method consisted of sitting the child with the head inclined slightly forward so fluid will flow out of the nose into a beaker held by the child under the chin; Ringers lactate solution (total volume 15 ml) is then injected in small amounts into the anterior nasal cavity, and aspirated repeatedly, via a glass nozzle until all 15 ml have dripped out of one side of the nose into the beaker. This same fluid is then used to wash out the opposite side of the nose.

Treatment of specimens. 0·2 ml of 1% sodium azide was added to each 10 ml of fluid obtained to halt bacterial and enzyme activity. Specimens were centrifuged at 4000 rev for 30 min in an ECCO bench centrifuge and the supernatant concentrated to 1 ml by negative pressure dialysis at 4°C. Specimens were stored at −20°C. Protein content was determined by measurement of optical density at 280 mμ assuming that the extinction coefficient of this mixed solution of proteins was 10.

Determination of IgA, IgG and albumin levels. This was done by immunodiffusion into antibody containing agar as described by Mancini, Carbonara & Heremans (1965) but three changes were made in this technique in order to adapt it to the determination of low levels of protein. The final agar concentration was reduced from 1·5% to 1%, 5 μl instead of 2 μl was delivered by micropipette into each punched hole in the agar and the antibody concentration used for determination of albumin was reduced to 1 : 20 and for IgA and IgG

* Behringwerke-AG, Marburg-Lahn.

to 1 : 40. Standard dilutions of normal human sera and colostral IgA (secretory IgA) were prepared by dilution with 1% bovine serum albumin in phosphate buffered saline (PBS). Determination of IgE was carried out by the radio immunodiffusion method of Rowe (1969) using a final antiserum concentration in the agar of 1 : 2000. The concentration of IgE in some preparations necessitated increasing the anti-IgE concentration to 1 : 400 and quantitation at this concentration was achieved by initial comparison of an IgE concentrate at various dilutions against WHO normal human sera.

A check of the protein loss in the concentration process was made by measuring the protein value before and after dialysis. By carefully washing the collodion membrane at the termination of concentration, the loss could be kept to between 10% and 30%. A check was also made of the possibility that there might be differential loss of IgA, IgG or albumin, but no disproportionate loss of these proteins was found. The possible differential loss of IgE was not checked.

RESULTS

The levels of IgA, IgG, albumin and total protein values of the nasal washing specimens from eighty-five normal children and fifty asthmatic children showed a considerable variation of total protein content. The concentrations of albumin, IgA and IgG were expressed as a percentage of the total protein in each specimen. This method of quantitation was essential, rather than an expression of the total value, as the washing technique inevitably varied in efficiency depending on the co-operation of the child. The results of these determinations are shown in Table 1. It should be noted that there is a considerable variation in the levels of all three proteins in both groups of patients as shown by the standard deviation values. However, there is no statistical difference between the mean values obtained for normal and asthmatic children.

The results of IgE values in nasal washings are also expressed in relation to the total protein as ng/ml of nasal wash protein. The results are shown in Table 2. The IgE content of some samples was in excess of that of the standards and could only be estimated by extrapolation of the standard curve of the IgE concentrate. No statistical analysis is necessary to demonstrate the considerably higher values (five–six-fold) found in asthmatic in contrast to normal children. One child (case No. 50) who had been seen in the surgical ward recovering from a burn was originally in the control group. The value of IgE was so high for a control patient, however, that the case was re-examined. The case records contained no reference to asthma. Conversation with the child's mother obtained the information that although the mother considered him entirely normal, he had considerable wheezing on exertion in the evenings, as did one of his three sibs (also considered 'normal'). This child was therefore transferred to the 'asthma' group.

DISCUSSION

This investigation has clearly shown that IgE is present in the nasal secretions of both normal and asthmatic children whose ages span the range 6–14 years. Ishizaka & Newcomb (1970) have also reported the presence of IgE in nasal secretions and sputum of both asthmatic patients and normal subjects. However, it is not possible to conclude from their data whether any statistically significant difference exists between the IgE levels in the exocrine

TABLE 1. Levels of albumin, IgA and IgG in nasal washings expressed as a percentage of total protein. Eighty-five normal children compared to fifty asthmatic children (ages 6–14)

	Mean (%)	Standard deviation	Standard error	*P* value
% Albumin				
Controls	17·9	13·3	1·44	>0·05
Asthmatics	18·5	13·7	1·93	
% IgA				
Controls	9·53	4·34	0·470	>0·05
Asthmatics	11·2	6·90	0·976	
% IgG				
Controls	4·55	2·47	0·267	>0·05
Asthmatics	4·23	2·65	0·374	

TABLE 2. Levels of IgE, expressed as ng/mg of nasal wash protein in twelve normal children and nineteen asthmatic children

Controls			Asthmatics		
No.	Age/yr	IgE ng/mg	No.	Age/yr	IgE ng/mg
1	6	46	1	14	750
3	10	54	5	6	325
5	13	25	7	11	600 plus
9	7	145	10	10	475
15	12	72	11	10	390
16	11	145	15	7	750 plus
24	8	72	17	12	660 plus
26	12	43	19	13	36
33	8	10	20	8	700 plus
37	6	46	21	12	700 plus
45	7	150	23	10	160
53	8	10	24	10	600 plus
			25	8	660 plus
			27	11	260
			28	11	850 plus
			39	7	660
			50	8	475
			53	10	300
			55	8	200

Controls average 68 ng/mg; Asthmatics average 400 ng/mg.

TABLE 3. Ratios of immunoglobulins in serum and nasal washings of asthmatic and control children

	Asthma			Control		
Source	IgE/IgG	IgE/IgA	IgA/IgG	IgE/IgG	IgE/IgA	IgA/IgG
Serum*	$1{\cdot}4\times10^{-4}$	$6{\cdot}7\times10^{-4}$	$2{\cdot}1\times10^{-1}$	$3{\cdot}3\times10^{-5}$	$1{\cdot}9\times10^{-4}$	$1{\cdot}8\times10^{-1}$
Nasal washings	$9{\cdot}5\times10^{-3}$	$3{\cdot}6\times10^{-3}$	2·7 (1·7*)	$1{\cdot}5\times10^{-3}$	$7{\cdot}1\times10^{-4}$	2·1 (2·6*)

* Calculated from Ishizaka & Newcomb (1970).

fluids of the two groups of subjects. The results reported in this paper clearly show that asthmatic children have higher concentrations of IgE in nasal washings than do normal children of the same age group. The average concentration of IgE in nasal washings of twelve normal children was 68 ng/mg protein and for the group of nineteen children with asthma was 400 ng/mg protein. This represents approximately a six-fold increase. In the latter group values in excess of 700 ng/mg protein were sometimes recorded. The fact that one child (case No. 50), originally placed in the control group but subsequently shown to be asthmatic, had an IgE level of 475 ng/mg nasal secretion protein is strong supporting evidence that this trend towards elevated IgE concentrations in asthmatic secretions is a real phenomenon. It is not altogether unexpected that such high values should be found in children with asthma, who are predominantly allergic in type.

IgE concentrations in the sera of normal individuals average 0·2–0·3 μg/ml. (Johansson, 1967, 1968; Ishizaka & Newcomb, 1970) but higher concentrations (Ishizaka & Newcomb, 1970) 1·39–1·59 μg/ml (Johansson, 1967) are found in allergic asthmatic patients. The average serum concentrations of IgG in asthmatic and normal children are 9·83 and 9·06 mg/ml respectively and for IgA 2·08 and 1·62 mg/ml respectively (Ishizaka & Newcomb, 1970). It is not possible to calculate the absolute physiological concentrations of these immunoglobulins in exocrine fluid but the ratios of IgE/IgG and IgE/IgA provides insight into source of IgE in exocrine fluid. In Table 3 comparison is made between the ratios of IgE/IgG, IgE/IgA and IgA/IgG in serum (data from Ishizaka & Newcomb, 1970) and nasal secretions (this publication). The IgE/IgG ratios in nasal secretions of asthmatic individuals are greater than those in serum by a factor of 67 and for normal children of the same age group by a factor of 45. If the IgE in nasal secretions is derived from serum by transudation during allergic reactions one would expect similar IgE/IgG ratios to occur in both serum and nasal secretions. That such is clearly not the case confirms the proposal of other investigators (Newcomb & Ishizaka, 1970; Tada & Ishizaka, 1970; Donovan *et al.*, 1970; Arturson *et al.*, 1969) that IgE is produced locally in the mucosa. Tse *et al.* (1970) reported some correlation between the presence of nasal IgE antibodies to ragweed allergen with P-K titres of the sera. Some of the nasal IgE antibodies could therefore be derived from serum by transudation but clearly this is not the major source of exocrine IgE.

Our findings indicate that there is no statistically significant difference between the concentrations of secretory IgA and IgG in nasal washings of asthmatic and normal children. In this respect they agree with the results of Ishizaka & Newcomb (1970) and Smith, Bellanti & Chanock (1967) but differ from those reported by other workers (Cohen *et al.*,

1970; Alford, 1968; Rossen *et al.*, 1965). Variations in technique and the subjects' ages probably account for these differences. However, the IgA/IgG ratio in nasal washings is 12–13 times greater than that in serum for both asthmatic and normal children. This is to be expected if secretory IgA in the external secretions is locally produced (Tomasi *et al.*, 1965).

Lichtenstein *et al.* (1968) have attributed to secretory IgA, in nasal secretions of patients with allergic rhinitis, blocking antibody activity to ragweed allergen. The fact that blocking antibody titres in these patients are considerably elevated in concentration above that of normal nasal secretions (Lichtenstein *et al.*, 1968), yet the total secretory IgA concentration is constant, contrasts with the situation for IgE in our asthmatic patients. In these patients the IgE levels in nasal secretion are elevated six-fold. While a proportion of this IgE may be accounted for from reaginic antibody directed against specific allergens of significance in causing asthma, it remains to be established what other specificities, if any, may be attributed to the remainder of the IgE in nasal secretions.

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