

Secretory and humoral immunologic response of atopic and non-atopic individuals to intranasally administered antigen

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Summary

Groups of twelve asymptomatic atopic and fourteen non-atopic individuals were immunized with tetanus toxoid 7.5 Lf units $\times$ 2 by means of nasal droplets. Serum and nasal secretion samples were collected on days 1, 15 and 36. Protein content of dialysed secretions was determined by the Lowry method and 1.0 mg/ml samples used for specific antibody assays by passive haemagglutination (PHA) and radioimmunosorption (RISA) employing cyanogen bromide activated Sephadex G-25. IgG, IgA, IgM, and IgD were quantitated by single radial diffusion (SRD), and IgE by the RISA technique.

Mean PHA secretion titres rose significantly in both groups during the course of the study. Serum and nasal secretion RISA IgA antitoxoid levels were higher in the non-atopic group throughout the course of immunization but both groups showed a significant increase in mean levels following immunization ($P < 0.05$). Conversely nasal secretion IgE-antitoxoid levels were significantly higher in the atopic group throughout the course of immunization although no quantitative increase in levels was noted.

Total IgG, IgD, IgE, and IgM levels in serum and secretions did not rise during the course of immunization but nasal wash IgA levels rose in both groups. IgD levels were significantly higher in serum and nasal secretions of the atopic group.

Our results suggest that atopic and non-atopic individuals differ quantitatively in Ig class specific antibody production following topical mucous membrane immunization.

Introduction

One salient feature of atopic respiratory disease is the ability of the atopic as opposed to the non-atopic individual to mount an IgE type response to small amounts of natural inhalant antigens, such as ragweed pollen, which come in contact with mucosal surfaces. Studies of the immunologic response of atopic and non-atopic individuals

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to previously unencountered, purified protein and polysaccharide antigens have indicated no marked differences in their development of humoral antibody and type I weal and flare skin reactivity after parenteral immunization via the intramuscular and subcutaneous routes (Leskowitz & Lowell, 1961; Salvaggio & Leskowitz, 1965; Schwartz & Terr, 1971). In contrast to these findings, intranasal immunization with these antigens induces type I weal and flare skin reactivity more readily in the atopic subject (Salvaggio *et al.*, 1964; Salvaggio, Kayman & Leskowitz, 1966; Bernstein & Safferman, 1973). These observations provide experimental evidence that one major difference between the atopic and non-atopic individual lies in their manner of handling antigens coming in contact with mucosal tissues. They might be explained by several possibilities including the following. (1) Group differences in local 'protective' secretory IgA which might influence antigen absorption across mucosal surfaces; (2) group differences in local IgA/IgE cell ratios reflected by differences in specific IgE and IgA class-specific antibody production in external secretions; (3) abnormalities in 'mucosal permeability' in the atopic individual; or (4) abnormalities in local antigen 'handling' or degradation.

It has been shown that there is no deficiency of secretory IgA *per se* in the atopic individual (Hobday, Cake & Turner, 1971; Salvaggio *et al.*, 1973) and that antigens can be readily absorbed across respiratory and gastrointestinal mucosal tissues in both atopic and non-atopic subjects (Walzer, 1942; Karakitsos, Salvaggio & Mathews, 1974). Little is known, however, concerning the comparative local immunoglobulin class-specific immune response of atopic and non-atopic individuals.

In this study such a comparative response to intranasally administered tetanus toxoid was determined to gather information concerning possible qualitative or quantitative group differences. This antigen was chosen because it had been previously shown to induce a local secretory response in man without ill effects, and was unlikely to have come into contact with respiratory tract mucosal tissues.

Materials and methods

Subjects studied

The subjects studied were medical and dental student volunteers. They were tested by scratch and intradermal skin testing, using 1:20 and 1:2000 dilutions respectively, for type I weal and flare skin reactivity to a battery of common local inhalant allergens (Ragweed, English plantain, Marsh elder, Johnson grass, Bermuda grass, cat dander, house dust, *Fusarium*, Live Oak, Pecan and *Helminthosporium*). On the basis of these tests, together with personal and family histories, they were assigned to the atopic (twelve individuals) or control (fourteen individuals) group. All atopic individuals had a positive history of past or present respiratory allergic symptoms and prominent type I skin reactivity to many of the allergens employed for scratch and intradermal skin testing.

Experimental protocol

Subjects were immunized intranasally with tetanus toxoid on day 1, followed by a 'booster' immunization on day 15. With the head tilted back at a 45° angle, 0.5 ml of tetanus toxoid containing 7.5 Lf units (Eli Lilly Co.) was placed into each nostril by means of droplets from a 1.0 ml syringe and the subject instructed to keep his head in this position for at least 3 min. To further ensure adequate antigen-mucosal contact,

each subject was instructed not to expel any nasal secretions for a further 30 min after instillation of the toxoid.

Baseline, pre-immunization samples of serum and nasal secretion were collected on day 1 and 'pre-booster' samples on day 15 before instillation of the toxoid. A final blood and nasal secretion sample was collected on day 36. Samples of nasal secretion were collected as previously described (Salvaggio *et al.*, 1973) and treated as follows: samples were homogenized, centrifuged at 3000 rev/min for 30 min at 4°C to remove particulate material and dialysed to exhaustion against distilled water in a 4°C cold room. Dialysed material was lyophilized, reconstituted with 1 ml of phosphate buffer (pH 8.9; 0.3 M) and stored at -20°C. To facilitate a comparison between samples, secretions were tested for protein content by the Lowry protein method (Lowry *et al.*, 1951) and working samples containing 1 mg protein/ml were prepared.

All serum and nasal secretion samples were tested utilizing the following procedures. (1) Passive hemagglutination in microtitre plates using tanned sheep red cells to which tetanus toxoid was conjugated; (2) specific tetanus antitoxoid quantitation by a modification of the radioimmunosorption assay technique (RISA) (Wide, 1969); and (3) immunoglobulin quantitation by the single radial diffusion (SRD) method using 1% agarose in barbiturate buffer (pH 8.2, 0.1 M) (Salvaggio, Arquembourg & Sylvester, 1970) or, in the case of IgE quantitation and measurement of IgM in secretions, by radioimmunosorption assay.

For the RISA technique, Sephadex G-25 superfine was activated with cyanogen bromide (Axen, Porath & Ernback, 1967) and 0.1 ml of tetanus toxoid or antiserum was added to 200 mg of the activated Sephadex. This mixture was then treated as described in the original method. After final washing, the conjugate was stored for assay at 4°C for immediate use or at -20°C for repeat analyses.

Anti-immunoglobulin antibodies (anti IgG, IgA, IgM, Behring Diagnostics, produced in rabbits; anti IgD and IgE, Meloy Labs, produced in goats) were radioiodinated by a modification of the method of McConahey & Dixon (1966) by reacting 0.06 ml of the antiserum with 4 mCi of ^{125}I . To initiate the reaction, 20 µg of chloramine T was allowed to react for 1 min at room temperature, then the reaction was stopped by addition of 100 µg of sodium metabisulphite. The labelled antiserum was chromatographed on a 20 × 1.5 cm column of Sephadex G-150 to remove unconjugated iodine and the first eluant peak was pooled for use.

For the RISA procedure, 100 µl of Sephadex-antigen conjugate at a dilution of 1 mg/ml in assay buffer was mixed with 25 µl of serum or nasal wash working sample. The tubes were incubated overnight at room temperature on a rotator at 200 rev/min. All conjugates were washed 3 × with assay buffer to remove unreacted serum or secretion. Labelled antibody, 100 µl, was then added and the tubes incubated overnight at room temperature on the rotator. The complex was again washed 3 × with assay buffer to remove unreacted labelled antibodies. For counting, the complex was washed into a counting vial with two 5 ml washes of Triton-X cocktail and counted twice for 10 min each on a Unilux I (Nuclear Chicago) scintillation counter. Controls of the conjugate and buffer were treated as above to measure non-specific absorption. All tests and controls were run in duplicate. Tests using 5, 10, 25, and 50 µl of pooled sample were run to ensure that volume of sample had no effect upon the test. Ten duplicate tests on a pooled sample showed that the test gave good reproducibility. To check for the specificity of labelled antibodies, a serum from a 3-month-old

hypogammaglobulinaemic child was tested with each labelled anti-immunoglobulin used and gave results comparable to those obtained with the Sephadex-conjugate controls.

Results

Because of the positive skew of the experimental values obtained, all data were subjected to log transformation before performance of further statistical evaluation by analysis of variance.

Passive haemagglutination titres

Mean PHA serum and nasal secretion anti-tetanus toxoid titres with standard error are listed in Table 1. There was no statistically significant difference between the

Table 1. Mean PHA anti-tetanus toxoid titres ($\log_2$) in atopic and non-atopic individuals immunized intranasally with tetanus toxoid

Sample	Group	Day 1	Day 15	Day 36
Serum	Atopic	10.7 (± 0.5)	11.3 (± 0.5)	11.1 (± 0.5)
	Non-atopic	10.0 (± 0.9)	9.9 (± 0.8)	10.2 (± 0.7)
Nasal secretion	Atopic	1.1 (± 0.6)	3.3 (± 1.0)	6.3 (± 1.2)
	Non-atopic	0.1 (± 0.1)	0.4 (± 0.3)	4.8 (± 0.9)

Parentheses = standard error.

atopic and control groups throughout the course of the study. No significant rise in PHA anti-tetanus toxoid titres was noted in the sera of either group but the rise in nasal secretion titres from pre-immunization levels was significant in both groups ($P < 0.0005$).

Quantitation of immunoglobulins

Pre-immunization serum and nasal secretion IgD levels were significantly higher in

Table 2. Mean immunoglobulin levels in serum of atopic and non-atopic individuals immunized intranasally with tetanus toxoid

Group	Immunoglobulin	Day 1	Day 15	Day 36
Atopic	IgG	9.7 (± 0.2)	9.5 (± 0.2)	9.3 (± 0.2)
Non-atopic	mg/ml	9.1 (± 0.2)	9.3 (± 0.2)	8.9 (± 0.2)
Atopic	IgA	2.76 (± 0.21)	3.06 (± 0.16)	2.81 (± 0.14)
Non-atopic	mg/ml	3.23 (± 0.21)	3.19 (± 0.22)	3.22 (± 0.25)
Atopic	IgM	0.63 (± 0.07)	0.48 (± 0.02)	0.61 (± 0.06)
Non-atopic	mg/ml	0.60 (± 0.09)	0.63 (± 0.12)	0.66 (± 0.07)
Atopic	IgE	623.3 (± 81.1)	617.5 (± 58.6)	642.9 (± 105.8)
Non-atopic	ng/ml	614.6 (± 73.2)	683.2 (± 62.9)	737.1 (± 58.9)
Atopic	IgD	52.1 (± 15.6)	57.7 (± 17.6)	64.4 (± 16.4)
Non-atopic	μ g/ml	30.5 (± 10.9)	31.4 (± 10.7)	29.8 (± 9.0)

Parentheses = standard error.

the atopic group ($P < 0.01$ for serum and < 0.05 for nasal secretions) (Tables 2 and 3) but there were no group differences in serum IgG, IgA, IgM and IgE levels. In nasal secretions IgA and IgM levels increased following topical immunization, and IgA levels remained consistently higher (though not statistically significant) in the non-atopic group throughout the course of immunization. There were no group differences in mean nasal secretion IgG, IgM and IgE levels (Table 3). IgD levels, although higher

Table 3. Mean immunoglobulin levels in nasal secretions of atopic and non-atopic individuals immunized intranasally with tetanus toxoid

Group	Immunoglobulin	Day 1	Day 15	Day 36
Atopic	IgG	0.023 (± 0.005)	0.036 (± 0.013)	0.016 (± 0.001)
Non-atopic	mg/ml	0.036 (± 0.008)	0.019 (± 0.002)	0.027 (± 0.005)
Atopic	IgA	0.26 (± 0.05)	0.32 (± 0.08)	0.37 (± 0.06)
Non-atopic	mg/ml	0.36 (± 0.05)	0.45 (± 0.04)	0.46 (± 0.05)
Atopic	IgM	1471.7 (± 149.8)	1526.7 (± 105.3)	1786.7 (± 190.9)
Non-atopic	ng/ml	1530.7 (± 158.9)	1577.9 (± 142.6)	1870.7 (± 233.1)
Atopic	IgE	142.5 (± 30.3)	174.6 (± 55.2)	180.4 (± 51.7)
Non-atopic	ng/ml	116.8 (± 19.2)	120.7 (± 19.4)	218.6 (± 37.3)
Atopic	IgD	53.4 (± 13.5)	47.4 (± 10.9)	50.0 (± 13.0)
Non-atopic	μ g/ml	27.1 (± 3.82)	24.1 (± 3.68)	27.9 (± 3.84)

Parentheses = standard error.

in serum and nasal secretions of the atopic group, did not rise in either group during the course of immunization.

Determination of anti-tetanus toxoid antibodies

Results of serum and nasal secretion RISA anti-tetanus toxoid determinations in atopic and non-atopic subjects are illustrated in Tables 4 and 5. Serum IgG and IgA

Table 4. Comparative mean serum anti-tetanus toxoid antibody (ct/min measured by RISA) in atopic and non-atopic individuals immunized intranasally with tetanus toxoid

Group	Immunoglobulin	Day 1	Day 15	Day 36
Atopic	IgG	1775.1 (± 201.1)	1987.6 (± 215.5)	2239.1 (± 231.5)
Non-atopic		2454.5 (± 281.2)	2699.4 (± 214.3)	2990.1 (± 258.2)
Atopic	IgA	1659.6 (± 356.7)	1940.3 (± 342.0)	2723.2 (± 447.2)
Non-atopic		2671.6 (± 393.2)	2966.3 (± 385.5)	3305.9 (± 471.2)
Atopic	IgM	893.6 (± 120.6)	1300.0 (± 421.5)	2170.9 (± 764.0)
Non-atopic		1394.4 (± 209.9)	1600.0 (± 186.6)	2160.4 (± 620.7)
Atopic	IgE	1142.9 (± 140.3)	1206.7 (± 143.0)	2242.8 (± 636.6)
Non-atopic		1295.1 (± 130.4)	1448.2 (± 103.8)	1795.0 (± 341.3)

Parentheses = standard error.

anti-toxoid antibody levels were significantly higher ($P < 0.05$) in the non-atopic group throughout the course of immunization although both groups showed a

Table 5. Comparative mean nasal secretion anti-tetanus toxoid antibody (ct/min measured by RISA) in atopic and non-atopic individuals immunized intranasally with tetanus toxoid

Group	Immunoglobulin	Day 1	Day 15	Day 36
Atopic	IgG	613.8 ($\pm$ 183.0)	793.3 ($\pm$ 231.4)	1156.2 ($\pm$ 321.1)
Non-atopic		548.9 ($\pm$ 124.9)	886.4 ($\pm$ 192.5)	1152.1 ($\pm$ 215.6)
Atopic	IgA	962.6 ($\pm$ 124.7)	1363.7 ($\pm$ 188.4)	1779.5 ($\pm$ 460.7)
Non-atopic		1534.9 ($\pm$ 290.8)	1676.4 ($\pm$ 187.2)	2289.8 ($\pm$ 230.4)
Atopic	IgM	526.3 ($\pm$ 138.5)	680.8 ($\pm$ 185.0)	822.3 ($\pm$ 209.2)
Non-atopic		676.6 ($\pm$ 190.4)	713.1 ($\pm$ 158.3)	1046.7 ($\pm$ 231.6)
Atopic	IgE	663.1 ($\pm$ 154.5)	699.5 ($\pm$ 141.5)	717.8 ($\pm$ 181.8)
Non-atopic		338.1 ($\pm$ 79.7)	316.9 ($\pm$ 66.9)	330.4 ($\pm$ 82.9)

Parentheses = standard error.

significant rise in mean levels ($P < 0.01$) during the course of immunization. Serum IgM-anti-toxoid levels were initially higher in the non-atopic group but rose significantly in the atopic group ($P < 0.01$) resulting in similar group mean levels following immunization. Mean serum IgE-anti-toxoid levels were similar in both groups prior to intranasal immunization but, following the booster immunization, there was a significant increase ($P < 0.01$) in the atopic group.

In nasal secretions there was no significant difference in IgG-anti-toxoid levels between the two groups but both showed a significant rise in levels during the course of immunization. Nasal secretion IgA-anti-toxoid levels were higher in the non-atopic group throughout the course of immunization but both groups showed a significant increase in mean levels ($P < 0.05$).

Quantitation of serum and nasal secretion samples for IgD-anti-tetanus toxoid activity revealed no difference between counts obtained for the samples and the Sephadex-tetanus toxoid controls, indicating no detectable IgD-anti-toxoid activity.

Discussion

Our current results suggest that atopic and non-atopic individuals differ quantitatively in Ig class-specific antibody production following intranasal immunization with tetanus toxoid antigen. Non-atopic subjects demonstrated higher IgA-anti-tetanus toxoid levels in nasal secretions and serum throughout the course of intranasal immunization. A significant rise in IgA and IgG nasal wash anti-tetanus toxoid antibody levels was, however, noted in both groups following immunization. Conversely, atopic individuals demonstrated higher nasal wash IgE-anti-tetanus toxoid antibody levels throughout the course of immunization although no significant rise in levels was noted following immunization.

Immunoglobulin D, G, and E levels in serum and nasal secretions did not rise during the course of immunization, but nasal wash IgA levels rose significantly in both groups. IgD levels were also higher in serum and nasal secretions of the atopic group but no specific IgD-anti-tetanus toxoid antibody was detectable. Our finding of higher IgD levels in the serum of atopic, as compared with non-atopic, individuals is in agreement with two previous reports (Berg & Johansson, 1969; Kohler & Farr, 1967), although other investigators have noted no group differences (Brasher, Hall & Bourland, 1973). The current finding of higher IgD levels in nasal secretions of atopic

individuals has not been previously reported and suggests further evaluation of the role of this immunoglobulin in exocrine secretions. At this time, any hypotheses for our observed group differences in IgD levels would be highly speculative since the function of IgD is unknown and it does not contain specific antibody activity for most antigens.

The finding of elevated serum IgE levels has been well established in atopy although considerable individual variability exists and some investigators have noted normal levels in many atopic subjects. The similar serum and nasal secretion IgE levels noted in our current study may reflect our use of asymptomatic study subjects, and our performance of tests during the first 2 months of the year, the time when atopic individuals in this area are least symptomatic. In this regard it has been demonstrated that serum IgE levels in asthmatic children increase with severity of symptoms (Hogarth-Scott *et al.*, 1971) and that serum IgE levels in atopic persons decrease from maximal values to normal levels following cessation of local pollination seasons (Kumar, Newcomb & Hornrook, 1971; Yunginger & Gleich, 1973). Our comparatively high IgE levels obtained for nasal secretions are difficult to explain and may reflect a relative indiscriminatory ability of the Sephadex technique when low levels of IgE are quantitated. In this regard, Polmar, Waldmann & Terry, (1973) have shown that when low serum IgE levels are measured using bromacetyl cellulose or Sepharose-bound anti-IgE, apparently false high IgE levels are obtained when compared with the more sensitive and reproducible double antibody radial immunoassay. In the study reported here Sephadex G-25 was used for conjugation and it seems likely that our unexpectedly high IgE levels may be due to a lack of sensitivity when this conjugate is used to measure the low levels of IgE in nasal secretions.

Our finding of higher specific IgA-anti-tetanus toxoid levels in nasal secretions of non-atopic individuals may provide an important clue in defining a 'protective' role for secretory IgA antibodies in atopy. By either failing to mount a sufficient local specific secretory IgA response against small quantities of inhaled antigen, or by other mechanisms such as possessing a structural mucous membrane defect or deficiency in local antigen handling the atopic individual might not bind or eliminate sufficient allergens locally to prevent stimulation of IgE producing cells. Evidence against these possibilities has been provided by Turk and co-workers who studied the ability of secretory IgA to inhibit antigen E-induced histamine release in non-atopic and ragweed sensitive atopic subjects and detected no significant group differences (Turk, Lichtenstein & Norman, 1970). Group differences in serum concentrations of exocrine IgA between atopic and non-atopic subjects which might reflect structural mucous membrane abnormalities have also not been detected (DeVald & Newcomb, 1972). In addition the distribution of immunoglobulins, including IgE, as demonstrated by immunofluorescent and radiographic studies of mucosal biopsy specimens is similar in atopic and non-atopic subjects (Callera *et al.*, 1971).

In support of this 'local abnormality' concept, however, are other reports of serum IgA deficiency in a significantly higher proportion of individuals with atopy and autoallergic disease in comparison with the population at large (Cassidy *et al.*, 1969; Amman & Hong, 1970; Schwartz & Buckley, 1971; Kaufman & Hobbs, 1970), the finding of elevated specific IgE nasal secretion antibody predominantly in atopic individuals (Johansson, 1967; Berg & Johansson, 1971; Kumar *et al.*, 1971; Hogarth-Scott *et al.*, 1971; Arbesman, Tse & Wicher, 1970; Dolovich, Tomasi & Arbesman, 1970), the known higher incidence of anti-milk protein antibodies in atopic subjects

(Rothberg & Farr, 1965), the reported association between transient IgA deficiency and infantile atopy (Taylor *et al.*, 1973) and the recent finding that repeated intranasal challenge with allergens leads to persistent reduction of symptoms in atopic individuals (Taylor & Shivalkar, 1972). Other significant recent animal model studies support this general hypothesis in revealing that local oral immunization can interfere specifically with uptake of macromolecular protein antigens across mucosal tissue of both germ free and conventional animals (Walker, Isselbacher & Bloch, 1972). The possibility of abnormalities in local antigen handling by atopic individuals is also worthy of consideration in view of the recent findings of significantly decreased antigen uptake by neutrophils and eosinophils of ragweed sensitive atopic subjects as compared to normal controls (Santilli *et al.*, 1973).

If our current findings of quantitative differences in local production of Ig class-specific antibody between atopic and non-atopic individuals are ultimately verified, they may reflect important group differences in the ratio of IgA and IgE producing cells associated with respiratory tract tissues. There is some evidence for local production of IgE as well as IgA in exocrine fluids, and contact of inhaled allergens with local respiratory tract IgE producing cells might be facilitated in atopic individuals based on their inability to mount an adequate local specific IgA antibody response.

Further studies are clearly needed to determine if local differences exist in IgE/IgA cell ratios, in antigen 'handling', or in response to previously unencountered synthetic antigens, among atopic and non-atopic subjects. Studies of the absorption and local handling of intrinsically labelled microbial antigens would also provide interesting leads toward answering these questions.

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