PAPERS AND ORIGINALS

IgE Antibodies to *Dermatophagoides pteronyssinus* (House-dust Mite), *Aspergillus fumigatus*, and β -Lactoglobulin in Sudden Infant Death Syndrome

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British Medical Journal, 1975, 1, 357-360

Summary

The prevalence of serum IgE antibodies to *Dermatophagoides pteronyssinus* (house-dust mite), *Aspergillus fumigatus*, and bovine β -lactoglobulin was significantly greater in cases of sudden infancy death (S.I.D.) than in a control group of infants of the same age range. This difference was most pronounced with *D. pteronyssinus* antibodies, which suggests that hypersensitivity to house-dust mite may be a factor in the aetiology of S.I.D. in Western Australia. Both the S.I.D. and control infants had similar serum concentrations of immunoglobulins G, M, and E but IgA levels were significantly higher in the control group.

Introduction

The number and diversity of theories proposed over the past two or three decades to explain the epidemiological and pathological findings in cases of sudden infant death (S.I.D.) suggest that this syndrome has a multifactorial aetiology. The characteristic appearance of the lungs at necropsy and the rapidity of death in apparently healthy infants have prompted the suggestion that anaphylaxis may be a contributing factor. The hypothesis that the anaphylactic reaction is based on hypersensitivity to cows' milk has received enthusiastic and continued support (Gold and Godek, 1961; Gunther, 1972) but so far has yielded, apart from animal model experiments (Parish, *et al.*, 1960), results which at most are equivocal (Gold and Godek, 1961; Parish *et al.*, 1964; Johnstone and Lawry, 1966). The fact

that house dust is one of the most important causative agents in bronchial asthma and allergic rhinitis prompted Mulvey (1972) to speculate that S.I.D. could be caused by anaphylaxis resulting from inhalation of *Dermatophagoides pteronyssinus* (house-dust mite). The heavy infestation of mites in the bedding of S.I.D. victims and the strong family history of atopy lend support to this hypothesis (Mulvey, 1972).

Measurement of total serum IgE, the major antibody carrying reaginic activity in man (Ishizaka and Ishizaka, 1967), in S.I.D. victims is likely to be unrewarding since IgE levels in the first two years of life are intensely variable (Johansson *et al.*, 1972). On the other hand, quantitation in the sera of S.I.D. victims of IgE antibodies with specificity for a range of allergens offers the most likely avenue for establishing a hypersensitivity (Type I) factor in cot death.

Morbid anatomical and histological examination supplemented evidence of blood gas analysis to suggest that death occurred from asphyxia due to pulmonary anaphylaxis (Hilton and Turner, 1974). The aim of the present paper is to extend that study to report measurements of IgE antibodies to *D. pteronyssinus*, *Aspergillus fumigatus* extracts, and β -lactoglobulin in the sera of S.I.D. victims and selected controls.

Materials and Methods

Subjects.—This paper forms part of a continuing study in Western Australia of the characteristics and possible aetiological factors in S.I.D. which is described elsewhere (Hilton and Turner, 1974). It comprises analyses of serum from 32 S.I.D. victims for whom samples were available in 1973 and 12 who came to necropsy during January to June, 1974. IgE antibodies to *D. pteronyssinus* were measured in sera from the 44 S.I.D. victims and 71 controls; slightly fewer S.I.D. samples (37) were assayed for their content of IgE antibodies to *A. fumigatus* and β -lactoglobulin. It is accepted that there is no fully satisfactory control group for S.I.D.; the present study included in this category sera from 14 infants dying from trauma or of "explained" causes (Hilton and Turner, 1974) and 57 living infants admitted to the Princess Margaret Hospital for surgery. The age ranges of infants in the three groups were: S.I.D. 2 weeks

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to 18 months, dead controls 3 weeks to 23 months, and living controls 1 to 45 months.

Allergen Preparations.—Freeze-dried *A. fumigatus* culture obtained from Greer Laboratories Inc., North Carolina, was extracted with a mixture of dimethyl sulphoxide (50% in water) and 0.1M sodium bicarbonate for three days at 4°C (Turner *et al.*, 1975 a). The mixture was then centrifuged, dialysed against 0.05M ammonium bicarbonate, and lyophilized. Bencard Prick/Scratch-Testing solutions of *D. pteronyssinus* 1.2% were used as a source of house-dust-mite allergens. Bovine β -lactoglobulin A, batch number 63513, was obtained from Koch-Light Laboratories, London.

Allergen-insoluble Complexes.—Allergens were coupled to Merck cellulose (microcrystalline, batch number 2590499) which had previously been activated by treatment with cyanogen bromide (Wide *et al.*, 1967 a). Coupling was carried out in 0.1M sodium bicarbonate as described by Wide *et al.* (1967 b). The optimum quantities of allergen extracts to be coupled were determined in separate experiments using known positive sera containing high levels of IgE antibodies to the appropriate allergens (Baldo *et al.*, 1975; Turner *et al.*, 1975 b). The amounts used with 100-mg quantities of CNBr-activated cellulose were: *A. fumigatus* extract 7.5 mg, *D. pteronyssinus* extract 6 mg, and β -lactoglobulin 5 mg.

Labelled Anti-IgE.—Immunoadsorbent-purified rabbit antibody specific for the Fc portion of human IgE and labelled with ^{125}I to a specific activity of approximately 4.4 $\mu\text{Ci}/\mu\text{g}$ was obtained from Pharmacia, Uppsala (batch number 2200).

The detection of specific IgE antibodies in sera was carried out according to the method of Wide *et al.* (1967 b). Test serum samples or samples of cord serum (50 μl) were mixed with 0.5 ml of a suspension of the allergen-cellulose complex (1mg/ml) in 3-ml Falcon plastic tubes and rotated overnight at room temperature. After washing three times with a phosphate-buffered saline pH 7.5 containing 0.5% Tween 20 and 0.2% bovine serum albumin ^{125}I -anti-IgE (40 000 c.p.m.) was added to each tube. After a further 12 hours of rotation the tubes were washed six times and the radioactivity remaining was measured by counting each tube for five minutes in a Packard Auto-gamma spectrometer. Samples were scored positive when the radioactive uptake was at least one and a half times that of negative controls—that is, cord serum.

Estimation of Immunoglobulin Levels in Sera.—The concentrations of IgE in sera were measured by the radioimmunosorbent test (R.I.S.T.) of Johansson *et al.* (1968). In the estimation Phadebas IgE test radioimmunoassay kits (Pharmacia) were used. Results were expressed as U/ml (Rowe *et al.*, 1970; Bazaral and Hamburger, 1972). Immunoglobulins G, A, and M were measured by radial immunodiffusion in Hyland plates.

Results

IgE antibodies to *D. pteronyssinus* were found in 23 (52.3%) of the 44 S.I.D. cases, 1 (7.1%) of the 14 dead controls, and 9 (15.8%) of the 57 live children with no obvious history of allergy. The high level of positive results in the live control group prompted further investigation. The presence of serum IgE antibodies to *D. pteronyssinus* was related to age, in that the youngest child who yielded positive results in the live control group was 15 months of age, whereas the S.I.D. cases spanned the age range 2 weeks to 18 months.

The numbers of subjects with IgE antibodies to *D. pteronyssinus*, *A. fumigatus*, and β -lactoglobulin in the age ranges 2 weeks to 19 months and 20 to 45 months are shown in table I. Of the live control children two out of 32 aged 19 months or less and seven out of 25 aged over 19 months had antibodies to *D. pteronyssinus*. This increased prevalence of IgE antibodies with age was most pronounced in the older age group. Of the eight children aged 31 to 45 months five had serum IgE antibodies to *D. pteronyssinus*, four to β -lactoglobulin, and one to

A. fumigatus. Since there was no apparent difference in antibody titre between the dead and the live controls these two groups are combined in table II, which shows the fractions and percentages of positive titres for children under 20 months of age.

TABLE I—Presence of Serum IgE Antibodies as a Function of Age in S.I.D. and Control Groups

Group	≤ 19 months of Age			> 19 Months of Age		
	<i>D. pteronyssinus</i>	<i>A. fumigatus</i>	β -Lactoglobulin	<i>D. pteronyssinus</i>	<i>A. fumigatus</i>	β -Lactoglobulin
S.I.D.	23/44	11/37	9/37	0/0	0/0	0/0
Control:						
Dead	1/10	1/10	1/10	0/4	1/4	0/4
Live	2/32	3/32	1/32	7/25	3/25	5/28

TABLE II—IgE Antibodies in Sera of S.I.D. and Control Infants less than 20 Months of Age

Infants	<i>D. pteronyssinus</i>		<i>A. fumigatus</i>		β -Lactoglobulin	
	No.	%	No.	%	No.	%
S.I.D.	23/44	52.3	11/37	29.7	9/37	24.3
Control (dead + live)	3/42	7.1	4/42	9.5	2/42	4.8
S.I.D. v. controls	$\chi^2 = 18.66$; $P < 0.005$		$\chi^2 = 3.99$; $P < 0.05$		$\chi^2 = 4.76$; $P < 0.05$	

* χ^2 test using Yates's correction.

The prevalence of IgE antibodies to all three allergens was significantly raised in the sera of S.I.D. victims when compared with the control group of the same age range. This increase was most pronounced with *D. pteronyssinus* ($P < 0.005$).

Stenius and Wide (1969) adopted a more stringent requirement for positivity in terms of IgE antibodies—namely, that the radioactivity uptake should be at least twice that of the negative controls—that is, cord serum. The numbers of sera with IgE antibodies to the three allergens taking as positive titres various ratios of radioactive uptake of test to cord serum are shown in table III. With the adoption of Stenius and Wide's (1969) criterion there is still a significant ($P < 0.005$) increase in the number of sera from S.I.D. victims compared with controls with IgE antibodies to *D. pteronyssinus*. The trends noted earlier with *A. fumigatus* and β -lactoglobulin antibodies, however, were not significant under these conditions.

TABLE III—Number of Sera from S.I.D. Victims and Controls of 20 Months of Age with IgE Antibodies at Different Levels of Positivity with Regard to Uptake of Radioactivity from Cord Serum

IgE Antibody Titres	No. of Sera with Positive Titres					
	<i>D. pteronyssinus</i>		<i>A. fumigatus</i>		β -Lactoglobulin	
	S.I.D.	Control	S.I.D.	Control	S.I.D.	Control
$1\frac{1}{2}$ –2 $\times$ cord titre	6	1	4	2	3	1
2–3 $\times$ cord titre	7	1	3	2	3	0
3–4 $\times$ cord titre	6	0	2	0	3	0
$> 4 \times$ cord titre	4	1	2	0	0	1
Total No. of subjects	44	42	37	42	37	42
S.I.D. v. controls	$\chi^2 = 12.425$; $P < 0.005$		$\chi^2 = 2.629$; N.S.		$\chi^2 = 3.107$; N.S.	

* χ^2 test using Yates's correction.

Of the 37 S.I.D. victims whose sera were assayed for the presence of IgE antibodies to all three allergens 20 yielded positive results to at least one (table IV). Of these, 8 (40%) gave a positive reaction to one allergen only, 8 (40%) to two allergens, and 4 (20%) to all three. By contrast all eight of the positive sera from the 42 controls which were also tested against the three allergens showed IgE antibodies to one allergen only.

The total serum IgE levels of the S.I.D. infants increased steadily with age though the individual values at each age

TABLE IV—Number of Infants Less than 20 Months of Age with Serum IgE Antibodies to One or More Allergens

Infants	1 Allergen	2 Allergens	3 Allergens	Total*
S.I.D.	8	8	4	20/37
Control (dead + live)	8	0	0	8/42

*Includes only those sera which were tested against all three allergens.

showed considerable scatter around the mean (fig. 1). There appeared to be no significant difference between the serum IgE levels of S.I.D. infants and those of the combined control group who were less than 20 months of age. One child in the control group, a boy of 19 months, had a serum IgE level of 650 U/ml but did not yield positive titres for IgE antibodies to any of the

FIG. 1—Serum total IgE levels S.I.D. (●) and control (○) infants as function of age.

three allergens. The relationship between total serum IgE and the presence of IgE antibodies to the three allergens in the S.I.D. victims is shown in fig 2. A positive regression was noted only with β -lactoglobulin antibodies.

FIG. 2—Relationship between total serum IgE levels and presence of IgE antibodies to *D. pteronyssinus*, *A. fumigatus*, and β -lactoglobulin in sera of S.I.D. victims.

Immunoglobulins G, A, and M were quantified in the sera of 44 S.I.D. victims and 42 controls, all of whom were less than 20 months of age. Of these infants 35 (80%) of the S.I.D. and 16 (37%) of the controls were 6 months of age or less. Since serum immunoglobulin concentrations increase with the age of the infant it was considered necessary to restrict comparisons between the two groups to the age range which encompassed most of the S.I.D. victims. For infants of 6 months of age or less there was no significant difference between the levels of serum IgG, IgM, and IgE, but IgA was significantly ($P < 0.05$) raised in the control group (table V).

TABLE V—Mean Concentrations of Serum Immunoglobulins in S.I.D. and Control Children 6 Months of Age or Under

Group	Immunoglobulin Class			
	IgG (g/l)	IgA (g/l)	IgM (g/l)	IgE (U/ml)
S.I.D.	6.22 $\pm$ 3.89	0.275 $\pm$ 0.242	0.810 $\pm$ 0.763	15.7 $\pm$ 14.1
Control	7.50 $\pm$ 4.04	0.411 $\pm$ 0.237	1.23 $\pm$ 1.36	15.2 $\pm$ 11.6
Significance	N.S.	$P < 0.05$	N.S.	N.S.

Discussion

Though clearly no single aetiological factor can be found to explain S.I.D. our work provides evidence to support the hypothesis that anaphylaxis may be one of the causative agents. Anaphylaxis in man may be initiated via several different pathways which have in common the release of pharmacologically active amines which induce smooth-muscle contraction and increased vascular permeability. The most common of these pathways is the interaction of antigen with specific IgE antibodies fixed to mast cells and circulating basophils. Though the fetus has the capacity to synthesize IgE at 11 weeks of gestation (Miller *et al.*, 1973) the concentration of this immunoglobulin in cord serum is low. Soon after birth active IgE synthesis begins but it is some time during the first months of extrauterine life before neonatal IgE fixes to and therefore sensitizes target cells in tissue (Stevenson *et al.*, 1971). We were unable to show any significant difference in the levels of serum IgE between the S.I.D. and control infants. The IgE concentration in both groups rose steadily during the first year of life, which is in general agreement with the results reported for normal children by Johansson *et al.* (1972). Serum IgE levels are significantly raised above the normal range in older children and adults with allergic disease—for example, asthma, hay fever, etc. (Turner *et al.*, 1974). The presence of relatively normal IgE levels in the sera of S.I.D. infants, however, does not necessarily exclude the possibility that an immediate hypersensitivity is involved in the syndrome. This follows from the fact that IgE is predominantly a cytotropic antibody and the levels of the immunoglobulin in postneonatal serum may not accurately reflect its concentration on tissue cells (Aas and Johansson, 1971). The presence of detectable IgE antibodies in sera with undetectable or low levels of total IgE may merely be a reflection of the relative sensitivity of the two assay systems.

Even if anaphylaxis is one of the contributing factors of S.I.D. it is unlikely that these infants would all be hypersensitive to the same allergen since atopic subjects in any given community show a spectrum of clinical reactivity to a range of allergens. While the choice of allergens was therefore somewhat arbitrary the selection of *D. pteronyssinus* and *A. fumigatus* was made on the basis that these were likely to be among the major allergens present in the microenvironment of the infant of less than 12 months of age (Mulvey, 1972; Lumpkins *et al.*, 1973). β -Lactoglobulin, the major allergen of cows' milk (Kletter *et al.*, 1971), was selected as the third allergen since repeated attempts have been made to implicate hypersensitivity to cows' milk as one of the causative agents in this syndrome (Parish *et al.*, 1960; Gold and Godek, 1961; Gunther, 1972).

In this study the S.I.D. victims had a greater prevalence of serum IgE antibodies to all three allergens, particularly *D. pteronyssinus*, than did the controls. It is now widely accepted that positive R.A.S.T. results correlate closely with clinical hypersensitivity as measured by skin test reactivity and provocative inhalation (Stenius and Wide, 1969; Stenius *et al.*, 1971). On this basis it is reasonable to assume that anaphylaxis mediated via IgE antibodies to *D. pteronyssinus* in particular may be one of the contributing factors in S.I.D. The fact that a large proportion of the S.I.D. infants had IgE antibodies to more than one allergen lends additional support to the concept of a hypersensitivity reaction. This study, however, has not shown that the IgE antibodies exert a biological activity in vivo, a

point of some importance, as Stevenson *et al.* (1971) suggested that the IgE immunoglobulins of the newborn may be less able to fix to dermal cells or to participate in the anaphylactic process than those of the older infant. The infant at 3 to 5 weeks of age, however, reacts to anti-IgE with weal-and-flare skin responses (Stevenson *et al.*, 1971). It is therefore likely that the infant should have an efficient IgE cell-fixing and histamine-releasing system by 2 to 3 months of age, when the prevalence of S.I.D. is maximal.

The continued epidemiological and pathological findings in S.I.D. suggest that it has a multifactorial aetiology. Whether anaphylaxis induced by hypersensitivity to these or as yet other unidentified allergens can fully explain these deaths remains unproved. There is little doubt, however, that this study has shown by an in-vitro assay a significant difference between S.I.D. and control infants of the same age.

This work was supported by grants from the National Health and Medical Research Council, the Princess Margaret Children's Medical Research Foundation, and the Asthma Foundation of Western Australia. We are grateful to Miss E. Quinn, Miss J. Butler, and Mr. C. Raven for skilled technical help. The co-operation of the coroners, the Commissioner of Police, and the Commissioner of Public Health of Western Australia is gratefully acknowledged.

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Peak Expiratory Flow in Youths with Varying Cigarette Smoking Habits

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British Medical Journal, 1975, 1, 360-362

Summary

Measurements of peak expiratory flow (P.E.F.) were done on 195 boys arriving at a detention centre, and again eight weeks later at the time of their discharge. During this time they took much physical exercise, and cigarette smoking and drug taking were not permitted.

At the initial assessment there was an impairment of P.E.F. inversely proportional to the amount of cigarettes smoked which was statistically significant.

There was a significant improvement in P.E.F. between reception and discharge in all groups of boys with varying smoking habits, all groups except the heavy smokers achieving near normal results after eight weeks. The heavier smokers improved more than the non-smokers or light smokers, but this difference was not statistically significant. Suggested reasons for this improvement are discussed.

Introduction

This study was conducted in a detention centre on youths aged 16-20 years. Most (89%) were cigarette smokers. The youths

studied were delinquent boys, who before arriving at the centre probably smoke more and probably have less opportunity and enthusiasm for physical exercise than youths of the same age seen in the general population. Most are from social classes III-V.

The routine in the centre gives much emphasis to physical exercise, regular meals, and sleep. Smoking is not permitted during their stay, and this is enforced by rigid supervision. There is, thus, an unusual opportunity to show any change in ventilatory function that might be attributed to smoking by comparing results obtained on their arrival with those on their discharge some eight weeks later.

Method

At the medical examination on reception all boys who were likely to stay for at least eight weeks and who had no gross physical disablement and no past or present history of asthma were accepted for the study. They were recruited as they were received, consecutively over a period of six months. The aim was to study 200 boys but though 215 were recruited 20 were incompletely studied because they were transferred to other establishments or because of illness at the time of discharge. This left 195 boys with completed data.

The following data was collected from each boy on arrival at the centre (and again on discharge eight weeks later in the case of peak expiratory flow (P.E.F.) measurements): (a) physical characteristics—that is, age, height, and weight (stripped); (b) the average daily cigarette consumption in the three months before reception, by careful direct inquiry; (c) any abuse of drugs, including the smoking of cannabis. A boy was considered

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