

Latent anaphylactic sensitivity of infants to cow's milk proteins

Histamine release from blood basophils

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Summary

Approximately 25% of infants tested released >9% of their blood basophil histamine content in the presence of cow's milk proteins, indicating a degree or level of latent anaphylactic sensitivity to these allergens. Approximately 10% of infants show a considerably higher level of sensitivity (14–63% histamine release). These findings fulfil an essential tenet of the modified anaphylactic hypothesis for cot-death.

Introduction

The modified anaphylaxis hypothesis for cot-death (Parish *et al.*, 1960a; Parish, Barrett & Coombs, 1960b) states that the human infant could become anaphylactically sensitized to cow's milk following oral administration, then, should regurgitation of the stomach contents containing cow's milk occur during sleep, milk could be inhaled into the lungs (the shock organ) causing anaphylactic constriction of the bronchial muscles. Due to this occurring during sleep there would be no struggle to overcome the obstruction to air-flow, there would simply be periods of apnoea with final cessation of respiration and death would quietly ensue.

Although a convincing animal model has been produced in the guinea-pig (Devey *et al.*, 1976) it is difficult to find conclusive evidence in the human infant (Coombs & McLaughlan, 1982). However, if it could be shown that a latent anaphylactic sensitivity to cow's milk occurs in otherwise healthy infants this would provide direct support for the hypothesis.

Three possible ways of investigating the existence of latent anaphylactic sensitivity to cow's milk proteins in infants are, namely (a) skin testing infants with cow's milk proteins, (b) examination of infant sera for milk specific IgE or IgG (STS) antibodies and (c) testing for blood basophil histamine release following challenge with milk proteins. The experimental work reported here is concerned with this latter (c)

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approach which provides an indication of biological function and as far as we know has not previously been applied to paediatric studies. Approaches (a) and (b) are considered in the Discussion.

Materials and methods

Patients

These were infants and children, either in-patients or attending out-patient clinics who were being bled for routine diagnostic tests. They were divided by age into three groups:

Group I —4 to 26 weeks (fifty-four cases)

Group II —27 to 52 weeks (thirty-two cases)

Group III—>1 to 12 years (forty-three cases). Of these, twenty cases were aged between 1 and 2 years, sixteen were between 2 and 5 years and seven were between 5 and 12 years of age.

Blood

Venous blood was taken in Lithium heparin bottles and kept at 4°C until tested (usually with 4 hr of collection).

Milk

Pasteurized whole cow's milk was microfuged at 10 000 *g* for 5 min, the middle layer was removed and diluted appropriately (i.e., 1/2–1/8) with phosphate buffered saline (0.15 M, pH 7.2).

Anti-IgE

Sheep anti-human IgE (raised to myeloma PS and insolubly absorbed with light chains) was diluted 1/10 with phosphate-buffered saline (PBS)—this was found to be the optimal dilution for histamine release with adult blood.

Histamine release from whole blood

Aliquots of 250 μ l of blood were mixed with 50 μ l of diluent (PBS), diluted milk, or diluted anti-IgE and incubated with regular agitation at 37°C for 30 min. Seven hundred microlitres of cold PBS was then added and the tubes were centrifuged at 4500 rev/min for 5 min. The cell-free supernatant was removed and an equal volume of 0.8 M perchloric acid was added to precipitate the protein. After centrifugation the histamine content of the supernatant fluid was assayed. Total histamine content of whole blood was determined by diluting an aliquot of whole blood 1/4 with PBS, de-proteinizing with an equal volume of 0.8 M perchloric acid, centrifuging and then assaying the histamine content of the supernatant fluid.

Histamine assay

An automated spectrophotofluorometric assay was used (Technicon Autoanalyser, Industrial method No. 164–73E) with minor modifications based on the method of Evans, Lewis and Thompson (1973).

Milk- and anti-IgE-induced histamine release from whole blood is expressed as a percentage of the total available histamine present in the blood cells, calculated as follows:

$$\% \text{ Histamine release} = \frac{\text{Milk/anti-IgE induced histamine release} - \text{PBS control}}{\text{Whole blood histamine} - \text{PBS control}} \times 100$$

Table 1. Infants aged 4–26 weeks. Total blood histamine and percentage histamine release (%HR) following antigen challenge with cow's milk and anti-IgE.

Patient No.	Whole blood histamine (ng/ml)	%HR after challenge with		Clinical Condition
		Milk	anti-IgE	
16	29.6	63.0	0	Angioneurotic oedema
8	51.2	42.4	13.6	Hydrocephalus
7	26.6	36.4	NT	Meningitis
2	40	28.6	14.3	Feeding problems
18	88.8	23.6	9.6	Averted cot-death
15	28.8	22.6	35.5	Averted cot-death
52	27.2	14.8	25.9	Pyrexia? URTI*
22	68.8	10.1	5.8	Averted cot-death
39	45.6	9.6	3.8	Bronchiolitis
4	52	9.5	NT	Vomiting
38	42.4	9.3	16.3	Diarrhoea? Lactose intolerance
50	23.2	9.2	14.8	Diarrhoea and vomiting
20	52.8	8.9	14.3	Vomiting? Pyloric stenosis
46	42.6	6.6	NT	Screaming baby
41	44.8	6.4	31.9	Vomiting? Pyloric stenosis
13	72.8	6.2	6.2	Rhesus disease, asthma/bronchiolitis
43	33.6	5.7	17.2	? Pyloric stenosis
3	99	5.3	28.7	Failure to thrive
27	40	5.0	15	Diarrhoea and vomiting
45	43.2	4.5	13.6	Gastro-enteritis
36	36	4.4	0	Bronchiolitis
30	52.8	4.0	8.0	Bronchiolitis
25	53.6	3.8	11.3	Diarrhoea
44	27.2	3.8	7.6	Averted cot-death
31	56	3.7	14.8	Vomiting
29	53.6	3.6	NT	Failure to thrive
21	50.4	3.5	12.3	Vomiting, rash after cow's milk
35	76	3.5	15.3	Averted cot-death
23	101.6	3.5	18.4	Rectal bleeding
48	106.4	3.3	23.3	Failure to thrive
28	39.2	2.5	0	? Pyloric stenosis
49	85.6	2.2	4.4	Failure to thrive
24	59.2	1.9	24.1	Vomiting
19	68	1.5	10.4	Averted cot-death
5	51.2	0	17.6	Bronchiolitis
6	128	0	6.5	Down's Syndrome
9	40	0	0	RSV† bronchiolitis
10	16	0	NT	Failure to thrive
11	17.6	0	11.8	Gastrostomy
12	28.8	0	0	Averted cot-death
14	51.2	0	44.1	Failure to thrive
17	47.2	0	9.3	Failure to thrive
26	57.6	0	15.3	Neonatal fit
1	22	0	16.6	Sideroblastic anaemia
32	32.8	0	0	Averted cot-death
33	72	0	46.3	Wheezy cough
34	48.8	0	22	Vomiting? Pyloric stenosis
37	41.6	0	12.5	Bronchiolitis
40	42.4	0	6.8	Bronchiolitis
42	57.6	0	4.8	Congenital heart disease
47	48	0	8.7	Tachycardia
51	43.6	0	16.3	? Pyloric stenosis
53	56	0	20.3	Hernia for surgery
54	65.6	0	32.4	Apnoeic spells

* Upper respiratory tract infection

† Respiratory syncytial virus

NT = Not tested

Table 2. Infants aged 27–52 weeks. Total blood histamine and percentage histamine release (%HR) following antigen challenge with cow's milk and anti-IgE.

Patient No.	Whole blood Histamine (ng/ml)	%HR after challenge with		Clinical Condition
		Milk	anti-IgE	
57	30.4	15.2	0	Rectal pull-through
83	52	13.5	7.7	Well baby, sibling, cow's milk allergy
61	33.6	12.5	0	Asthma/eczema
67	41.6	11.1	0	URTI*
63	36	10	10	Hydrocephalus
58	46.4	9.4	20.7	Failure to thrive
68	40	9.3	32.6	Failure to thrive
85	12	9.1	9.1	Near drowning
56	32	9.1	NT	Sideroblastic anaemia
86	43.2	8.2	22.5	Convulsions
55	70	7.7	7.7	Bronchospasm
69	84	6.4	10.6	Cow's milk intolerance
62	100	5.2	38.3	Eczema
87	72	4.8	7.1	Asthma? Cow's milk allergy, diarrhoea
60	78.2	4.6	19.4	Failure to thrive
84	68.8	4.0	2.7	Diarrhoea and vomiting
70	84.8	2.1	12.8	Diarrhoea
71	47.2	2.1	6.3	Eczema
59	52.8	0	30.4	Colic
64	40.8	0	0	Pyrexia? Meningitis
65	121.6	0	0	Cystic fibrosis
66	39.2	0	5.3	Meningococcal septicaemia
72	114.4	0	1.5	Failure to thrive
73	56	0	23.7	URTI*
74	52	0	0	Bronchiolitis
75	43.2	0	NT	Failure to thrive
76	60	0	21.8	Failure to thrive
77	40.8	0	15	Failure to thrive
78	117.6	0	5.4	Failure to thrive
79	35.2	0	28.6	Febrile convulsions? URTI*, Eczema
80	40	0	0	Battered baby
81	96	0	3.6	Failure to thrive
82	48	0	0	URTI*

* Upper Respiratory Tract Infection

NT = Not tested

Results

The total blood histamine and percentage histamine release (%HR) from blood basophils after challenging aliquots of whole blood with cow's milk and anti-IgE are shown in Table 1 (infants aged 4–26 weeks), Table 2 (infants aged 27–52 weeks) and Table 3 (children aged > 1 year). Tables 1, 2 and 3 also give the clinical conditions of the patients at the time when the blood was taken. It was not possible to use blood from healthy babies since it could be considered unethical to bleed them specifically for this investigation.

Table 3. Children aged > 1 year. Total blood histamine and percentage histamine release (%HR) following antigen challenge with cow's milk and anti-IgE.

Patient No.	Whole Blood Histamine (ng/ml)	%HR after challenge with		Clinical Condition
		Milk	Anti-IgE	
89	120	73.4	63.5	Eczema
98	41.6	66.0	30.0	Vomiting
90	44	53.9	69.6	Atopic eczema and asthma
94	92	48.1	44.8	Eczema and asthma
96	104.2	24.3	0	Cerebral palsy
93	17.6	20	20	Asthma
97	26.4	17.9	17.9	Failure to thrive
99	17.6	14.3	0	Failure to thrive
105	31.2	12.9	9.7	Gastro-enteritis
114	32.8	11.8	14.7	Eczema and asthma
129	44.4	11.0	3.0	Multiple food allergy
88	66	10.6	16.7	Milk allergy
118	32	9.4	9.4	Asthma
121	34.4	6.7	10	Hypothyroidism
125	53.6	6.7	2.5	Eczema
110	42.4	6.4	12.8	Diarrhoea
111	87.2	6.3	12.6	Failure to thrive
101	92	5.7	19	Coeliac, Cow's milk intolerance
130	26	5.5	5.5	Diarrhoea
107	64	5.5	9.6	Meningitis
106	67.2	5.5	2.7	Behaviour disturbance
100	41.6	4.5	4.5	Allergic rash to cow's milk and beef
104	66.4	4.3	8.6	Pyrexia
92	120	4.1	3.4	Wheezy sibling of cot-death infant
91	152	3.8	38.9	Eczema and asthma
128	179.2	3.6	50.5	Eczema
103	32	3.0	12.1	Febrile convulsions
115	72.8	2.9	8.6	Asthma
109	96.8	1.8	8.2	Diarrhoea
120	69.6	1.4	4.2	Colic
124	104.8	0.8	5.6	Coeliac
123	128.8	0.7	2.0	Hyperactive
95	103.2	0	21.8	Eczema
102	43.2	0	4.8	Meningitis
108	111.2	0	4.0	Diabetic
112	59.2	0	13.8	URTI*
113	47	0	NT	Diarrhoea
116	67.2	0	9.7	? Allergy to milk/gluten
117	82.4	0	15.8	Bronchitis
119	45.6	0	9.1	Atopic, wheezy, bronchitis, eczema
122	64	0	0	Mumps, leukaemia
126	52	0	0	Failure to thrive, Diarrhoea and Vomiting
127	100.8	0	10.2	Eczema and asthma

* Upper Respiratory Tract Infection

NT = Not tested

Table 4. Numbers and percentages of cases within each group of patients showing either no histamine release or a definite histamine release after challenge with cow's milk or anti-IgE.

Group	Histamine release after challenge with			
	Cow's milk		Anti-IgE	
	0%HR	>9%HR	0%HR	>15%HR
I (4-26 weeks)	20/54 (37)	12/54 (22)	6/49 (12)	20/49 (41)
II (27-52 weeks)	15/33 (45)	9/33 (27)	8/31 (26)	10/31 (32)
III (>1 year)	11/43 (26)	13/43 (30)	4/42 (10)	12/42 (29)
III (>1 year excluding asthma and eczema)	8/30 (27)	7/30 (23)	4/29 (14)	5/29 (17)
III (>1 year, asthma and eczema cases only)	3/13 (23)	6/13 (46)	0/13 (0)	7/13 (54)

Percentages are given in parentheses.

Whilst in some patients no histamine release could be detected after challenge with milk, graded responses ranging from 1.5 to 63%HR (Table 1), 2.1 to 15.2%HR (Table 2) and 0.7 to 73.4%HR (Table 3) were found in the remaining patients. Since in this investigation it was not possible to include a control group of infants (i.e. healthy infants receiving breast milk only, from mothers on cow's milk-free diets) the response of a normal adult population to milk challenge was also measured. Out of eighteen normal adults (laboratory workers), we were never able to detect >9%HR after milk challenge. We have therefore arbitrarily taken any value of >9%HR to be a positive response to milk and regarded those from 0 to 9%HR to be of doubtful clinical significance. Similarly, the response of our normal adult donors to the dilution of anti-IgE used throughout this study was always >15%HR.

Table 4 shows the number of cases in each group which gave absolutely no detectable response to cow's milk and anti-IgE, and also those which gave a positive response to cow's milk and anti-IgE (i.e. >9% and <15%HR respectively).

Discussion

An essential tenet of the modified anaphylaxis hypothesis for cot-death is that a small number of otherwise normal infants must have a latent anaphylactic sensitivity to cow's milk and the object of the present investigation was to produce, if possible, direct and objective evidence for this using allergen-induced histamine release from blood basophils. Since healthy infants are not normally bled we used blood from hospitalized infants being bled for diagnostic purposes.

The test procedure has shown that a considerable number of infants released more than 9% of their total available histamine after incubation with an appropriate dilution of cow's milk. The children over 1 year of age were inevitably more selected with a bias towards asthma and eczema. In Table 4 therefore, this group has been divided to show the response of recognized atopic patients compared with other children of the same age. In Groups I, II and III, 22, 27 and 30% respectively released more than 9% of their blood histamine after challenge with milk. However, when the recognized atopics were

calculated separately from Group III it was found that 46% of atopics released more than 9% of their blood histamine compared to only 23% of non-atopics in Group III.

Allergen-induced histamine release from blood basophils under physiological conditions is a direct measure of a systemic latent anaphylactic sensitivity, although it does not take into account any preferential stronger sensitivity of the lungs themselves due to sensitization of local mast cells, and indeed it also excludes any local protective mechanisms which may exist. In another investigation at present in progress, we do in fact have evidence of local type I sensitivity in the gut without systemic blood basophil sensitivity. It is particularly interesting that some of the infants (especially in Groups I and II) showing this biological sensitivity, showed no obvious clinical indications of milk hypersensitivity, i.e. the sensitivity of their blood basophils to cow's milk was indeed 'latent'.

Other investigative procedures for revealing latent systemic sensitivity include skin testing and the measurement of IgE or IgG (STS) antibody. In the older literature using intracutaneous injections of milk into the skin of infants Du Bois, Schloss and Anderson (1925), found transient cutaneous hypersensitivity in 50% of both marasmic and normal infants following weaning onto cow's milk. Lippard (1939) reported positive skin tests in 35% of eczematous infants. Using Prausnitz-Küstner passive transfer skin-testing (Tallerman, 1934), reaginic antibodies to cow's milk proteins were demonstrated in the sera of infants recovering from gastro-enteritis. More recently, skin testing of infants and detection of serum IgE antibodies to milk by RAST have been studied by Dannaeus and Johansson (1979) and they found that 71% of infants with immediate onset of gastrointestinal symptoms to cow's milk had positive skin tests and positive RASTs. Serum IgE antibodies to β -lactoglobulin have been detected by RAST in the infants of atopic mothers but not in the infants of normal mothers (Dannaeus, Johansson & Foucard, 1978). Short-term sensitizing IgG antibodies (IgG-STS) have been measured by passive transfer into the skin of non-human primates, Pepys *et al.* (1979) found IgG (STS) antibodies to cow's milk in the sera of six out of fourteen atopic asthmatics who as children had infantile eczema. Parish (1971) found IgG (STS) in the sera of three out of nine children aged 10 to 30 months who had gastro-intestinal disorders 12–36 hr after ingesting cow's milk and he was also able to detect these antibodies in the sera from two cases of cot-death. Turner, Baldo and Hilton (1975) using RAST-detected IgE antibodies to β -lactoglobulin in nine out of thirty-seven cases of cot-death and two out of forty-two control infants. Anaphylactic sensitivity in infants to cow's milk undoubtedly occurs though the actual incidence is difficult to determine (Savilahti, 1981).

The RAST measures only IgE antibodies free in the serum and anaphylactic status is governed by the antibodies absorbed with strong affinity to mast cells and circulating basophils. In our opinion, the method we have used in the present investigation, namely the assay of histamine released from basophils in whole blood in the presence of the specific allergen (namely cow's milk), should provide a sensitive and direct indication of systemic sensitization.

Further investigations along the present lines are in progress with special attention to certain clinical categories. Infants presenting under the general heading 'neurological' and displaying >9% histamine release registered sufficiently frequently to deserve further attention. High titres of antibodies to cow's milk in the sera of mongoloid children were demonstrated by Nelson (1964).

In Group I we examined blood from eight cases of 'averted cot-death' (i.e. infants

resuscitated from a state of sudden respiratory collapse). Milk-induced histamine release from three of these cases was appreciable (10–24%HR). The time at which the blood sample is taken after such an episode could be very important if an anaphylactic reaction is involved, since a state of anti-anaphylaxis with greatly reduced histamine releasing capacity could exist for many hours after a generalized reaction.

In the modified anaphylaxis hypothesis, we have presumed that the offending allergen is cow's milk, but this does not have to be the case. Other possible allergens include Respiratory Syncytial Virus and the house dust mite *Dermatophagoides pteronyssinus* Turner *et al.*, 1975). These allergens are now being investigated for allergen-induced histamine release.

Whilst the present investigations were focused primarily on the anaphylactic hypothesis of cot-death, the information gained has broader relevance in providing an objective measurement of the type I allergic status of infants and young children. With a freshly taken heparinized blood sample the allergic profile can be measured and the offending allergens identified (and other allergens excluded; often equally important). Sequential studies would be of great value to determine whether sensitization is transient or persistent. It was interesting to find in the older children in Group III that those with an atopic history released greater amounts of histamine in the presence of anti-IgE than non-atopic children. Similar findings have been reported with adults (Ishizaka *et al.*, 1969). However, in the younger children in Groups I and II atopic infants were less frequently recognized clinically although >15%HR after anti-IgE challenge was frequently found (i.e. 41% of Group I and 32% of Group II). It is possible that these children represent a pre-atopic group and may well go on to become clinically recognized as atopic.

In conclusion, it seems that an essential tenet for the modified anaphylaxis hypothesis for cot-death—namely that a percentage of 'normal infants' have a latent anaphylactic sensitivity to cow's milk has been satisfied.

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