

Basophil histamine release in children with adverse reactions to cow milk

Comparison with RAST and skin prick test

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Basophil histamine release was examined in 26 children suspected of having cow milk allergy (CMA). Following oral challenge with cow milk, the initial adverse reaction reappeared in 20 children, the majority developing urticaria. The urticaria patients showed a high degree of correlation between the results of histamine test, RAST and skin test. Children with gastrointestinal symptoms reacted to milk challenge, but only a few showed a positive histamine test, RAST and skin test. Among the patients with atopic dermatitis, the tests gave mostly negative results, which was in accordance with the lack of response to a milk challenge. The results obtained by removal from and fixation to the cell surface of IgE indicate an IgE-mediated reaction in CMA, which, in connection with the correlation between histamine test and RAST or skin test, suggests basophil histamine release as a suitable method for testing Type I allergy in children suspected of CMA.

Key words: basophils; children; cow milk allergy; histamine release; IgE; RAST; skin prick test; Type I allergy.

CLINICAL ASPECTS

The results of our experiments performed on basophils indicate that IgE is important in allergic reactions to cow milk. These results and the highly positive correlation shown between the results of the histamine release assay and those of RAST and skin test suggest that the basophil histamine release test is a useful alternative for detecting the occurrence of cow milk-specific IgE.

In children with cow milk allergy (CMA) two types of adverse reactions can be identified. One group shows an immediate onset of symptoms (within 1 h) and the other a delayed reaction. Children with immediate reactions usually respond with urticaria, although gastrointestinal and other manifestations are also seen. The majority of these children have a positive skin test and positive RAST to cow milk (CM) (3, 8, 9, 11). In contrast, a negative skin test and RAST are normally found in children developing a delayed reaction, usually gastrointestinal symptoms (4, 5, 8, 19).

Since a Type I allergic reaction has been suggested in cases with a rapid onset of symptoms, an IgE-dependent mechanism was examined in

CMA suspected children by basophil histamine release test, and the results were compared with those of the RAST and skin test.

MATERIAL AND METHODS

Patients

The study comprised 26 children (19 boys and 7 girls) suspected of CMA. They had been consecutively referred to the Paediatric Department, University Hospital, Gentofte. Their mean age at the start of their symptoms was 4.7 (range 1-48) months and at the time of diagnostic

challenge 15 (range 1–63) months. A control group consisted of six non-atopic controls who showed no adverse reactions to CM. Their basophils liberated histamine following challenge with rabbit-antihuman IgE, which defined them as cellular responders, and they showed no histamine release following challenge with CM. The diagnoses of CMA were based on the following criteria: disappearance of symptoms after elimination of CM from the diet and reappearance of symptoms within 24 h of an oral challenge. In children with equivocal response, the oral challenge was repeated once.

Challenge procedure

The patients were hospitalized for the milk challenge procedures. The challenge was based on open studies, since a good correlation between an open and a placebo-controlled challenge has been obtained in young children (7).

The children remained on a diet free of milk and any other foods suspected of causing adverse reactions for at least 1 week before and during the challenge period. Usually the patients had been on such a diet for more than 4 weeks.

The following amounts of CM were administered. *Day 1*: 5, 10, 20, 30 or 60 ml given at intervals of 30–60 min; *day 2*: 120 ml as a single morning dose; *day 3*: 240 ml as a single morning dose, possibly followed by increasing doses up to a normal intake; and if necessary, *day 4*: a normal milk intake.

The challenge was considered positive if within 24 h of a given dose the child developed a recurrence of previous symptoms.

Allergen

Extract of raw CM for skin test, RAST and histamine release test was supplied by ALK, Copenhagen, Denmark. Rabbit-antihuman IgE (ϵ -chain) 400,000 IU/ml was from Behringwerke AG, Marburg, FRG.

Histamine release

Microfibre method. Venous blood (5 ml) was drawn into a plastic syringe and anticoagulated

with 0.25 ml 0.2 M EDTA (pH 7.2). The blood sample was washed 3 times with 15 ml Pipes-AMC (albumin, magnesium, calcium) (21) and resuspended in this buffered solution to a final volume of 5 ml. Fifty- μ l samples of this suspension were placed in glass microfibre-prepared microtitre plates (international patent application No. WO 83/00229). To avoid trapping of cells into the microfibrils, 50 μ l Pipes-AMC was added to the wells before addition of the washed blood cells. The samples were incubated for 60 min at 37°C with 20 μ l CM extract or anti-IgE, both in 10-fold dilutions. Released histamine was thus bound to the microfibrils (21). Interfering substances were removed by washing the plates for 1.5 min with deionized water. Histamine was released from the fibres by addition of 20 μ l HC10₄/OPT mixture (2 mg o-phthalaldehyde, Fluka, dissolved in 1 ml methanol and 1 ml 0.59% HC10₄). After 10 min at room temperature, 65 μ l 0.05 N NaOH was added and the coupling reaction was terminated after 10 min by addition of 65 μ l 0.59% HC10₄. The histamine content of each well was determined in a Farrand spectrophotofluorometer, and only a net release of histamine > 0.15 ng histamine, 2 HCl was considered significant (6).

Conventional leukocyte histamine release assay. Venous blood (10 ml) was drawn into a plastic syringe, anticoagulated with 0.5 ml 0.2 M EDTA (pH 7.2), and the leukocytes were isolated by the Ficoll-Hypaque gradient method, and thereafter washed and suspended in Tris-AMC (22). 45 μ l leukocyte suspension was incubated at 37°C for 40 min with 5 μ l allergen or anti-IgE. Allergens and anti-IgE were omitted for determination of the spontaneous histamine release and for determination of the total histamine content: in the latter case the samples were left at 4°C. Thereafter 3 ml ice-cold Tris-AMC was added and the samples centrifuged at 2000 *g* for 10 min. The supernatant was discarded and the residual histamine was determined in the cell sediment (22).

The histamine release was expressed as a percentage of the total histamine content of the sample. Since spontaneous histamine release

amounted to up to 10%, only a histamine release $> 10\%$ was considered significant.

Removal of IgE and passive sensitization of basophils. IgE was removed from the basophil cell surface by washing the blood and by suspending the blood cells in phosphate buffer (pH 3.6) for 5 min at 4°C. The IgE-deprived cells were then washed and resuspended in Pipes-AMC (6).

Passive sensitization of IgE-deprived cells was performed by incubating the cells with an equal volume of patient serum for 2 h at 37°C in a shaking bath. The cells were washed three times with Pipes-AMC at 250 g for 6 min and resuspended in this buffered solution.

Cellular responders and non-responders. To differentiate between cellular responders and non-responders, anti-IgE was used as the classical tool. Cells were incubated for 40 min at 37°C with the anti-IgE preparation in final dilutions of 10^{-3} to 10^{-5} . Histamine release indicates a cellular responder and no release a non-responder.

RAST. Specific IgE against CM was determined by RAST (26). Only allergy classes 3 and 4 were considered positive.

Skin test was performed as prick test with the raw CM extract. The response was estimated according to the Scandinavian Allergy Standard (1). Histamine (1 mg/ml) and sodium chloride (0.9%) were used as positive and negative controls. Wheals $\geq$ the size of the histamine wheal were regarded positive.

RESULTS

Histamine release from basophils by cow milk extract

Histamine release caused by CM extract was examined in basophils from children with suspected CM hypersensitivity. Fig. 1 shows the release of histamine as estimated by the conventional leukocyte histamine released assay (Fig. 1A) and by a new microfibre method for large-scale screening of Type I allergic reactions (Fig.

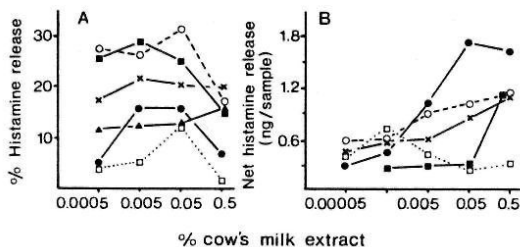


Fig. 1. Histamine release caused by cow's milk (CM) extract in cell suspensions from 11 children with suspected CM hypersensitivity. Allergy testing was performed by the conventional leukocyte histamine release assay (A) and by the microfibre method (B). Duplicate determinations.

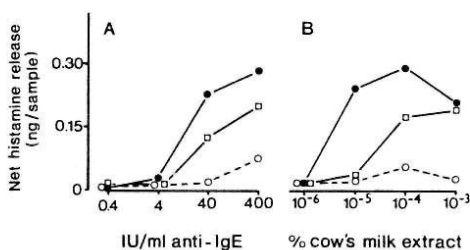


Fig. 2. Histamine release caused by anti-IgE (A) or CM extract (B) in cell suspensions from a child suspected of CM hypersensitivity. Intact cells (●—●), cells deprived of IgE (○—○) and with addition of IgE (□—□). A representative example from 8 patients. Duplicate determinations.

1B). Bell-shaped dose-response curves were often obtained with both methods. The maximal histamine release from the basophils of the patients varied between 13 and 30% of the total histamine content of the samples. Mostly, the maximal release was obtained in final dilutions of 0.0005 to 0.5% of the raw CM extract. No histamine release ($< 10\%$) was obtained in cells from the six controls.

The role of IgE in histamine release caused by milk allergen was examined by removal of IgE from the basophils prior to incubation with CM extract. Fig. 2 shows that removal of functional IgE, demonstrated by reduced or no response to anti-IgE (A), decreased or abolished the response to milk allergen (B). Similar results were obtained in all eight children tested. Further, refixation of IgE by passive sensitiza-

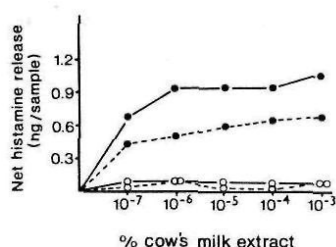


Fig. 3. Histamine release caused by CM extract after passive sensitization of IgE-deprived cells from a control. Passive sensitization by sera from 2 children with CM hypersensitivity (●) and from 2 normal individuals (○). Duplicate determinations. Note that in the absence of passive sensitization no histamine release was obtained in intact or IgE-deprived cells from normal individuals.

tion was performed in cells from three of the children by incubating the IgE-deprived cells with autologous serum. The histamine release induced by the milk allergen was reestablished by this procedure (Fig. 2).

The existence of an IgE-mediated reaction was further substantiated by similar experiments in which patients' sera were transferred to IgE-deprived cells from the controls. Fig. 3 shows that histamine release by CM extract was obtained after this passive sensitization with

sera from the patients but not with sera from the controls.

Examination of correlation between positive histamine release response, results of diagnostic tests and clinical symptoms

Only cellular responders can be tested directly for histamine release from their basophils. Therefore, the patients were divided into cellular responders, and non-responders by a positive or negative histamine release response to anti-IgE. Eighteen of 26 patients were verified as cellular responders and eight as non-responders. Accordingly, these eight children were unable to respond to a challenge of their cells with CM allergens. Allergy testing therefore also included examination of CM-specific IgE in serum by the indirect histamine release assay. Sera were all examined on the same day by passive sensitization of cells from the same person in the control group. To compare the direct histamine release assay with the indirect test, eight cellular responders showing histamine release by the direct test were also tested by passive sensitization. A histamine release was obtained in cells from all eight children by both tests. A positive histamine release response

Table 1

The relationship between histamine release, RAST and skin test to cow milk in 26 children suspected of CM hypersensitivity

	Patient No.	Symptoms by oral challenge	Histamine release	RAST	Skin test
*Challenge positive	1	anaphylaxis	+	+	+
	2-12	urticaria	+	+	+
	13		+	-	+
	14		+	-	-
	15		-	+	+
	16		-	+	-
	17	gastro-intestinal symptoms	+	+	+
	18		-	+	+
	19-20		-	-	-
*Challenge negative	21	asthma	+	+	+
	22	urticaria	-	-	-
	23	atopic dermatitis	+	+	+
	24-26		-	-	-

*Oral challenge

was therefore defined as histamine release obtained either by direct test or by passive sensitization. Table 1 shows that 17 of the 26 children responded with positive histamine release.

On oral challenge with CM, the initial symptoms reappeared in 20 of the 26 children (Table 1). Fifteen of 16 children with urticaria showed a positive response obtained by small amounts of milk. The reaction appeared within 1 h. An immediate response resulting in anaphylactic shock was also observed. No response was obtained in a child with bronchial asthma. Four children with gastrointestinal manifestations responded to larger amounts of milk with vomiting within 1 h and subsequent diarrhoea. Four children with atopic dermatitis did not respond.

In patients with suspected Type I allergy, such as urticaria, anaphylaxis, and asthma, the findings of a positive histamine release response were in agreement with those of the oral challenge test in 14 of 16 (88%) cases (Tables 1 and 2). Similar results were obtained using RAST and skin test (Table 2). Table 1 shows that the same individuals responded with histamine release and positive RAST or skin test in 75

and 81% of the cases, respectively. In contrast, the children with gastrointestinal symptoms showed no correlation between oral challenge and the other tests. Thus, all four children responded to the intake of CM, but only one showed a positive histamine release, while two had a positive RAST and a positive skin test (Table 2). Three of four patients with atopic dermatitis showed negative histamine release, RAST and skin test in accordance with their lack of response to milk intake (Table 2).

DISCUSSION

Adverse reactions to CM are frequent during infancy (13, 25) and seem to depend on various mechanisms. A Type I allergic reaction is suspected in cases with rapid onset of symptoms (14), and Type III or IV reactions in patients with a delayed onset of symptoms (15, 16, 18, 20). Further, various non-immunological mechanisms might be responsible for intolerance to CM (17).

In the Type I reaction, antigens react with IgE-antibody bound to mast cells and basophils, leading to release of histamine and other mediators. Histamine release thus indicates a Type I

Table 2
Relationship between histamine release test, RAST and skin test

		Urticaria, anaphylaxis, asthma					
		Histamine release		RAST		Skin test	
		+	-	+	-	+	-
Oral challenge	+	14	2	14	2	14	2
	-	1	1	1	1	1	1
		Gastro-intestinal symptoms					
		Histamine release		RAST		Skin test	
		+	-	+	-	+	-
Oral challenge	+	1	3	2	2	2	2
	-	0	0	0	0	0	0
		Atopic dermatitis					
		Histamine release		RAST		Skin test	
		+	-	+	-	+	-
Oral challenge	+	0	0	0	0	0	0
	-	1	3	1	3	1	3

reaction, and the basophil histamine release technique can be used as a sensitive method to detect Type I allergy (21-23). This method was used to examine the occurrence of Type I allergy in children with suspected CMA. Basophil histamine release was examined in leukocyte suspensions. One assay included the conventional leukocyte histamine release assay and the other, a new, simple microfibre method for large-scale screening of Type I allergic reactions (21, 22). In patients allergic to inhalant allergens, both assays were found to give almost identical results (21).

Histamine release was obtained when cells from most of the patients were challenged with CM extract, whereas this was not the case in the control group. In both assays bell-shaped dose-response curves were often obtained, which are well-known from Type I allergic reactions caused by inhalant allergens (22, 23).

An IgE-mediated reaction was illustrated by removal from and fixation to the cell surface of functional IgE-molecules. The removal was shown by little or no release of histamine from the cells after challenge with anti-IgE. In these IgE-deprived cells, CM extract caused little or no release of histamine. When, however, IgE was again bound to the cell surface by passive sensitization, the response to milk was regained. Passive sensitization with serum from children with CMA also caused a positive response in cells from the control group. These results indicate an IgE-mediated reaction to cow milk in children with CMA. The possibility of IgG₄ being involved seems unlikely, since the serum concentrations of IgG₄ antibodies specific to milk did not correlate with either the results of the provocation tests or the serum levels of milk-specific IgE antibodies (2).

It is well known that *in vitro* some children do not respond with basophil histamine release to a specific antigen or to anti-IgE, although they show Type I allergic symptoms to the antigen in question (12). This unresponsiveness of the basophils might be due to impaired IgE receptor complex activation or to some subcellular functioning rather than to a lack of cell-bound IgE (24). No response was obtained by milk allergens in such non-responders. However,

testing of these children was performed by using their sera for passive sensitization of cells from the control group. This could be done, since a positive histamine release response by the direct test always resulted in a positive histamine release response by passive sensitization.

Oral challenge with CM, provoked a recurrence of the initial symptoms in 20 of the 26 children suspected of CM hypersensitivity. The majority of the children developed urticaria, although some gastrointestinal symptoms were also seen. The majority of the children with urticaria showed positive histamine release response, and positive RAST and skin test to CM. The results of RAST and prick test are in agreement with other authors (3, 8, 9, 11). In contrast, only a minor part of the children with gastrointestinal symptoms showed positive histamine release, RAST and skin test. Others have obtained similar negative results with RAST and skin tests for IgE antibodies to cow's milk in intestinal CMA (4, 5, 10, 19). In the group of children with a negative challenge, the clinical pattern was dominated by atopic dermatitis, and the majority of these children showed negative histamine release response, and negative RAST and skin test.

The results of the histamine release assay were similar to those of the RAST and skin test regarding identification of IgE against CM allergens in children with suspected CM hypersensitivity. Further, as the results obtained by removal from and refixation to the cells of IgE indicate an IgE-mediated reaction in CMA, the basophil histamine release is suggested as a method for testing Type I allergy in children showing adverse reactions to cow milk.

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