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Basophil Histamine Release in Cord Blood

Regulatory Role of IgE

F. Ebbesen,[2] P. Stahl Skov,[1, 3] C. Jensen,[1] S. Norn[1] and I. Søndergaard[3]

[1]Dept. of Pharmacology, University of Copenhagen, [2]Dept. of Paediatrics, University Hospital of Glostrup, [2]Dept. of Gynaecology and Obstetrics, University Hospitals of Glostrup and Herlev, and [3] Laboratory of Medical Allergology, Medical Department TTA, State University Hospital, Copenhagen, Denmark

Thirty-two cord blood samples were studied for histamine releasing capability by using a sensitive glass microfibre-based histamine analysis. Histamine was obtained after challenge with anti-IgE in 24 of the 32 samples. However, the net release in cord blood was only 25% of that in adult blood and no relationship was found between histamine release response, total IgE in cord plasma, and a family history of atopic diseases. The low histamine release in cord blood seemed to be associated with the immunological IgE receptor complex activation and not with an immature basic cell function, since the calcium ionophore A23187 which bypasses the receptor complex induced identical histamine release curves in cord and adult blood. Furthermore, when comparing the results of passive sensitization of basophils from new-born and adult persons, the new-born basophils possessed a significant fraction of free IgE receptors, whereas in adults most of the receptors were occupied by IgE.

Key words: basophil; cord blood; histamine release; IgE; passive sensitization.

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Basophil histamine release from cord blood has been examined in a previous study and the results indicated that basophils from new-borns released only little histamine by challenge with anti-IgE (4). It is therefore possible that basophils from new-borns differ from the adult cells in their maturity. In the present study we therefore examined the basophil histamine release response in cord blood in the intact cells, passively sensitized cells, and cells from which IgE was removed, to obtain information on the fraction of free IgE receptors. Furthermore, the basic cell function was elucidated by the cell response to calcium ionophore.

New-born infants and adult subjects

This study included cord samples consecutively taken from 32 healthy new-born infants with a mean gestational age of 39 weeks (range 34–43 weeks) and a mean birth weight of 3260 g (range 1820–4430 g). Four of the infants were preterm (gestational age < 37 weeks): two infants had a gestational age of 34 weeks and the other two of 35 weeks. A family history of atopic diseases in first degree relatives (parents and/or siblings) was found in 10 infants (31%): atopic dermatitis in two (6%), allergic rhinitis in six (19%), and bronchial asthma in four (12%).

Blood samples from 16 adult, non-allergic, healthy individuals with an age range from 23 to 46 years were included.

Five ml of fresh cord and adult blood was anticoagulated with 0.5 ml 0.2 M EDTA (pH 7.2) and stored at 4°C for up to 10 h before use.

MATERIAL AND METHODS

For histamine release experiments grass pollen allergen from Pharmacia, Sweden (10,000 BE/

ml) was diluted in Pipes-AMC to final concentrations in the reaction mixture of 100, 10 and 1 BE/ml. Anti-IgE (400,000 U/ml) from Behringwerke (Germany) was used in final concentrations of 10^3, 10^2, 10, 1 and 10^{-1} U/ml. Calcium ionophore A23187 from Calbiochem (Switzerland) was used in final concentrations of 2, 1, 0.5, 0.25, and 0.125 μM.

For histamine release experiments Pipes-AMC, a buffered solution (pH 7.4) was used consisting of 10 mM piperazine-N,N (bis-ethane sulphonic acid), 140 μM sodium acetate, 5 mM potassium acetate, 0.6 mM $CaCl_2$, 1.1 mM $MgCl_2$, glucose 1 mg/ml, human serum albumin 0.3 mg/ml and heparin, Leo, Denmark, 15 IE/ml.

Preparation of cord blood

Intact cells. Cord blood (1.2 ml) was washed three times at 250 g for 6 min at room temperature with 9 ml Pipes-AMC and resuspended in this buffer to a final volume of 1.2 ml.

IgE-deprived cells. IgE was removed from the basophil cell surface by a modification of the method originally described for Ficoll-Hypaque isolated cells (8). Cord blood (1.2 ml) was washed three times at 250 g for 6 min at room temperature with 9 ml 0.9% NaCl containing human serum albumin 0.3 mg/ml. The cells were thereafter suspended in 9 ml NaH_2PO_4 buffer (pH 3.6) containing 145 mM NaH_2PO_4, 5 mM potassium acetate and human serum albumin 0.3 mg/ml and were incubated at 4°C for 5 min. After centrifugation (250 *g* for 6 min), the acid-treated cells, now devoid of IgE, were suspended in 9 ml Pipes-AMC buffer, washed three times (250 *g* for 6 min) and resuspended in this buffer to a final volume of 1.2 ml.

Passively sensitized cells. Both intact and IgE-deprived cells were sensitized with plasma from a grass pollen-allergic hay fever patient (the specific diagnosis was verified by case history, skin prick test, nasal provocation, RAST, and basophil histamine release to grass pollen). An equal volume (1.2 ml) of cells was incubated with an equal volume of plasma for 120 min at 37°C in a shaking bath. Thereafter the cells were washed three times at 250 *g* for 6 min with 9 ml Pipes-AMC and resuspended in this buffer to a final volume of 1.2 ml.

Basophil histamine release from cord blood by the microfibre method

The 32 cord samples were prepared as described above and 50 μl samples of these preparations were placed in glass microfibre-prepared microtiter plates (10) (US Patent No. 4,550,085). Pipes-AMC (50 μl) was added to the plates before addition of blood. The samples were thereafter incubated for 60 min at 37°C with 20 μl of grass pollen or anti-IgE in the final concentrations given above. The net release of histamine was calculated on the basis of a standard histamine curve, where blood samples were incubated with 0, 0.15, 0.30, and 0.6 ng histamine, 2 HCl. The spontaneous release of histamine was negligible, since almost identical standard curves were obtained when histamine was added to Pipes-AMC instead of to blood.

After incubation, released histamine was bound to the microfibres and interfering substances were removed by washing the plates for 1.5 min with deionized water. Thereafter histamine was released from the fibres by addition of 20 μl $HClO_4$/OPT mixture (2 mg o-phthaldialdehyde, Fluka, dissolved in 1 ml methanol and 1 ml 0.59% $HClO_4$). After 10 min at room temperature, 65 μl 0.05 N NaOH was added and the coupling reaction was terminated after 10 min by addition of 65 μl 0.59% $HClO_4$. The histamine content was determined in a Farrand spectrofluorometer provided with a microflow cell and an autosampler.

Basophil histamine release from isolated cord blood cells

In 17 cord and 16 adult blood samples basophil histamine release was performed by the conventional technique to obtain release of histamine expressed in per cent of the total histamine content of the sample. The leukocytes were

isolated by the Ficoll-Hypaque gradient technique (6, 7). Two hundred µl of the leukocyte suspensions were incubated in plastic tubes at 37°C for 40 min with anti-IgE or the calcium ionophore A23187 in the above-mentioned final concentrations. All concentrations were tested in duplicate. A23187 and anti-IgE were omitted for 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 Pipes-AMC was added to the samples and they were centrifuged at 2000 *g* for 10 min. The supernatant was discarded and the residual histamine was determined in the cell sediment. To the cell sediment was added 400 µl NaOH/OPT mixture (2 mg o-phthaldialdehyde, Fluka, dissolved in 1 ml methanol and 9 ml 0.05 N NaOH). After 4 min 400 µl 0.175 N H_3PO_4 was added and the samples centrifuged at 3000 *g* for 10 min. The histamine content was determined spectrofluorometrically in the supernatant in a Farrand provided with a micro-flow cell and an autosampler. Samples were read at 355/455 nm (excitation/emission). The release of histamine was expressed as a percentage of the total histamine content of the sample. In the absence of anti-IgE or A23187 the spontaneous histamine release amounted to 5–10% in all experiments.

Histamine content in basophils, number of basophils and percentage of eosinophils

Basophils in cord and adult whole blood and in the isolated leukocyte suspensions were stained and counted according to (6). The content of histamine per basophil was determined in the leukocyte suspension, and based on this content and the number of basophils in whole blood the basophil histamine content per ml blood was calculated.

Total immunoglobulin in cord blood plasma

IgE was determined by a method quantitating subnanogram quantities of IgE (5). IgA and IgM were determined by routine methods.

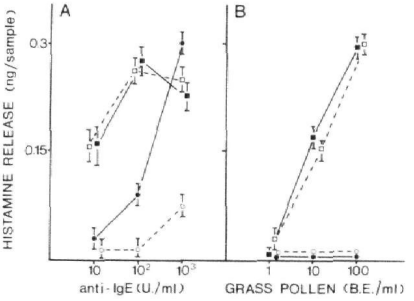


Fig. 1. Histamine release with anti-IgE (A) and grass pollen (B) from cord blood. Intact cells (● ——— ●). IgE-deprived cells (○ ------ ○). Intact cells sensitized with grass pollen plasma (■ ——— ■). IgE-deprived cells sensitized with grass pollen plasma (□ ------ □). Each point represent mean of 24 cord blood samples ± SEM. Note that in 3 cord samples the intact cells responded to grass pollen. These results are not included in the figure.

RESULTS

Histamine release from cord blood

Histamine release from 32 cord blood samples was performed using intact cells, IgE-deprived cells, and intact or IgE-deprived cells passively sensitized with plasma from a grass pollen-allergic patient. The samples were challenged with anti-IgE or grass pollen in different concentrations and released histamine was determined. Anti-IgE released histamine from intact cells in 24 of the 32 cord samples, and the dose-response curve for the responding cells appears from Fig. 1A. The histamine response was almost completely abolished in IgE-deprived cells, since the histamine release was depressed and a shift to the right of the dose-response curve was obtained (Fig. 1A). Both intact and IgE-deprived cells became equally sensitized to anti-IgE by passive sensitization with serum from a grass pollen-allergic patient, since almost identical curves were obtained by a shift to the left of the dose-response curves (Fig. 1A).

When cord blood was challenged with specific antigen (grass pollen) instead of anti-IgE, no histamine release was obtained from IgE-deprived cells (Fig. 1B). The same was true

for the intact cells (Fig. 1B) with the exception of three cord samples, where histamine release was obtained by grass pollen. In these three samples a pattern was obtained similar to that obtained by challenge with anti-IgE, i.e. removal of IgE from basophils caused no or little release of histamine by grass pollen, but the sensitivity of the cells to grass pollen was regained by passive sensitization. As above, intact and IgE-deprived cells became equally sensitized to grass pollen by passive sensitization with serum from a grass pollen-allergic patient, since almost identical dose-response curves were obtained (Fig. 1B).

By passive sensitization of intact cells, which responded to anti-IgE but not to grass pollen, 70% of the samples responded to grass pollen. When intact cells not responding to anti-IgE were sensitized to grass pollen, 80% responded to grass pollen. When IgE was removed from the cell surface before the passive sensitization, the same pattern was obtained.

Figs. 1A and B show that the maximal release of histamine in cord blood was 0.3 ng ± 0.02 (mean ± SEM) per 50 µl sample, which is in contrast to the release in 50 adult blood samples amounting to approximately 1.3 ng (results not shown), indicating that the net release in cord blood is approximately 25% of the release in adult blood.

Table 1

Examination of the relationship between total IgE in plasma, histamine release response to anti-IgE, and a family history of atopic disease

Total IgE (ng IgE/ml)	Number of histamine responses	
	Positive	Negative
0.2	13 (2)	5 (3)
0.3	3 (1)	2
0.4	3 (1)	
0.6	1	
0.8	1	
0.9	1 (1)	
1.3	1	
1.4		1 (1)
2.0	1 (1)	

Number of children with a family history of atopic diseases is given in parenthesis.

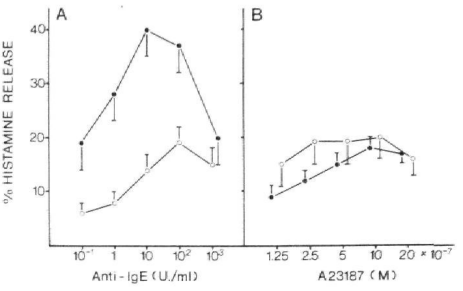


Fig. 2. Histamine release with anti-IgE (A) and the calcium ionophore A23187 (B) in Ficoll-Hypaque-isolated cells from 17 cord blood samples (○ ——— ○) and 16 blood samples from adult healthy volunteers (● ——— ●). Each point represents mean and SEM values.

Relationship between histamine release and plasma IgE in cord blood

Total IgE was measured in plasma from the 32 cord samples, and a possible relationship to anti-IgE-induced histamine release was examined. As appears from Table 1, there was no relationship, since 13 positive and five negative histamine responses were obtained in cord samples, all containing 0.2 ng IgE/ml plasma. Furthermore, one sample with high plasma IgE (1.4 ng/ml) showed no histamine release. Infants with a family history of atopic diseases did not correlate to histamine release response or to total IgE.

In the same 32 cord samples the plasma concentrations of immunoglobulins A and M were 0.01 (0.00–0.08) and 0.03 (0.00–0.08) g/l (median range), respectively. These concentrations for cord blood were normal (1, 2, 12), indicating that there was no contamination with maternal blood, either by materno-foetal transfusion or at the time of collection.

Histamine released by anti-IgE and A23187 in cord and adult blood

Seventeen cord and 16 adult blood samples were assayed by the conventional histamine release method using Ficoll-Hypaque-isolated cells. Fig. 2A shows that the percentage of histamine release (but also absolute release since the basophils of

adult persons contain more histamine) and the cell sensitivity to anti-IgE were lower in cells from cord blood compared with cells from adult blood. This is in contrast to histamine release by the calcium ionophore, where no statistical difference was observed between adult and cord blood histamine release.

Number of basophils and histamine content in cord and adult blood

Table 2 shows that the histamine content in cord blood basophils was approximately one third of the content in adult blood basophils. The number of basophils were, on the other hand, significantly higher than found in adult blood. Based on the histamine content per basophil and the number of these cells in blood, the histamine content per ml blood was calculated, and as can be seen from the Table 2, the histamine content in cord blood was half of that found in adult blood.

DISCUSSION

The present investigation was performed to investigate the capability of cord blood basophils to release histamine, and to examine factors of importance for the release. A prerequisite for performing such a study is a highly sensitive basophil histamine release assay which allows many tests to be carried out on small sample volumes. Such an assay has been developed in our laboratory and is based on specific adsorption of histamine to glass microfibres and subsequent fluorometric determination of histamine (9, 10). This assay, which allows 100 tests to be performed on 5 ml blood, has therefore been used in the study.

Table 2

Comparison between cord and adult blood

	Pg Histamine basophil	10^3 Basophils ml blood	Basophil histamine content in blood (mg/ml blood)
Cord blood	0.6 ± 0.1 (n = 16)	110 ± 24 (n = 16)	66
Adult blood	2.0 ± 0.2 (n = 16)	64 ± 9 (n = 16)	128

Cord blood basophils showed a capability to release histamine, since histamine release was obtained in 24 of 32 cord samples by challenge with anti-IgE. No relationship was found between histamine release response, total IgE in plasma and a family history of atopic diseases. Thus, at present it is not obvious that a positive histamine release reaction to anti-IgE is of any value in predicting the development of allergy. In the clinic a long-term follow-up of the neonates is in progress. The histamine release was smaller than in adult blood, since the net release in cord blood was only 25 % of that in adult blood. Our study indicates that at least two factors might contribute to the low net release of histamine. One of these factors is due to the low content of histamine in cord basophils (30 % of adult basophils) and therefore in cord blood, but not to a decreased number of basophil cells, which is nearly doubled (Table 2). These results are in accordance with Mendoza et al. (4). The other factor is caused by a low cellular releaseability to anti-IgE, since a 20 and 40 % histamine release were found in cord and adult blood, respectively (Fig. 2A). The low basophil cell releaseability might be associated with the immunological IgE receptor complex activation and not with an immature basic cell function, since the calcium ionophore A23187, which bypasses the receptor complex, induced almost identical histamine release curves in cord and adult blood.

A possible mechanism could be that the basophil cell surface in cord blood contains only a smaller fraction of IgE-receptors occupied by IgE, which means that the cell possesses a high proportion of free IgE receptors. That this is the case is seen by comparing the present results with our earlier findings in basophils from adult, normal individuals. When the cells were passively sensitized with plasma from a grass pollen-allergic patient and challenged with anti-IgE, an increase in cell sensitivity to anti-IgE was observed in cord blood (Fig. 1A) but not in adult blood (11) by a shift to the left of the dose-response curve.

This seems to indicate a higher proportion of free IgE receptors on cord blood basophils than on adult basophils. The suggestion is supported by similar experiments where the cells were challenged with specific antigen (grass pollen) instead

of anti-IgE. In cord blood, the intact cells mostly caused no histamine release by specific antigen. When these cells were passively sensitized, they obtained the ability to release histamine as by passive sensitization of the IgE-deprived cells, indicating a high proportion of IgE receptors on the intact cells. This is in contrast to adult blood, where the passive sensitization caused a higher histamine release and cell sensitivity in IgE-deprived cells than in the intact cells (11).

A substantial difference therefore exists between new-born and adult persons, since basophils from the new-borns possess a significant fraction of free IgE receptors, whereas in adults most of the receptors are occupied by IgE. It is possible that the fraction of free IgE receptors in cells from new-borns constitutes a potential for development of sensitized cells responsible for type I allergic reactions in childhood. In this context it is possible that the three new-born infants showing histamine release by specific antigen (grass pollen), probably by prenatal allergic sensitization (3, 13), might have a predisposition to allergy. The three infants were born in November. A transplacental passive sensitization in these infants was unlikely, since all three plasmas had a low content of IgE (0.2 µl/ml) and no immunoglobulin A and M. There were no indications that the mothers had grass pollen allergy, since the case history, RAST and histamine release response to grass pollen were negative.

Our data will be examined in the coming years for possible clinical relevance. All the infants tested will be followed for development of allergy to evaluate whether our individual results could be of predictive significance for allergic diseases in childhood.

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Address
Dr. *Per Stahl Skov*
Laboratorium for Medicinsk Allergologi
Afdeling TTA 7542
Rigshospitalet
Tagensvej 20
2200 København N
Denmark

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