

Sulphidoleukotriene release of cord blood basophils in response to allergen stimulation correlates with neither a family history of atopy nor a subsequent development of atopic eczema

C. Walaschek, A. Heinzmann, M. Weckmann and M. V. Kopp

University Children's Hospital, Freiburg, Germany

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Summary

Objective We tested a possible relationship between sulphidoleukotriene (SLT) release of cord blood (CB) basophils, a family history of atopy (HA) and subsequent development of atopic eczema.

Population and methods A cohort of 86 neonates were involved (48.8% males; 46.5% with a positive HA⁺). CB samples were analysed for *in vitro* SLT release quantified by ELISA, and in a subgroup for basophilic activation (CD 63 expression) by flow cytometry in response to a positive control (anti-IgE-receptor antibody), an allergen-mix (TOP and PTOP), egg white (EW), egg yolk (EY), and the purified allergens β -lactoglobulin (BLG) and α -lactalbumin (ALA).

Results Median concentrations of SLT were 124.2 (negative), 3871.5 (positive), 123.9 (TOP), 128.5 (PTOP), 113.1 (EW), 108.4 (EY), 125.2 (BLG) and 122.3 (ALA) pg/mL. Groups of HA⁺ and HA⁻ show no difference in all analysed allergens. An allergen-specific SLT release (defined as SLT >125 pg/mL above individual baseline and a stimulation index >2) was detected in 98% (positive control), 5% (TOP), 7% (BLG), 3% (ALA) and 2% (EW and EY), respectively. After a median observation period of 18 months, *n* = 7 out of 70 children developed an atopic eczema, but we observed no association between CB SLT release (positive response to at least one tested allergen).

Conclusion Allergen-specific SLT release is detectable in 15.5% of healthy neonates, irrespective of their family history of atopy. However, early allergen-specific SLT release is not predictive for the development of atopy.

Keywords allergy, atopic eczema, atopy, cord blood, sulphidoleukotrienes

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Correspondence:

Dr Matthias Kopp, University Children's Hospital, Mathildenstraße 1, D-79106 Freiburg, Germany. E-mail: Matthias.kopp@uniklinik-freiburg.de

Introduction

The prevention of atopic disorders is currently one of the challenges of contemporary medicine [1, 2]. There is a need to find predictive parameters to help us identify children at risk for the development of atopic eczema, asthma or allergic rhinitis (AR).

So far, a positive family history and cord blood (CB) IgE have been used as predictors for the development of atopic disorders [3–6]. However, the majority of atopic manifestations occur in infants with no demonstrable risk at birth, and the sensitivity and predictive value of CB IgE has not proved satisfactory [4, 6].

During the last decade, several attempts have been made to evaluate specific immunological parameters such

as proliferative capacity or cytokine release of cord blood mononuclear cells (CBMC) for their potential value in predicting atopic diseases. The proliferative capacity of CBMC in response to different nutritive and inhalative allergens generated much interest in the possibility that a prenatal contact of antigens primes the neonatal immune system [7–11]. Up to 100% of CBMC responded to the cow's milk protein β -lactoglobulin (BLG) [9, 10]. However, the quantification of proliferative response is not helpful in discriminating between atopy-prone and non-atopy-prone neonates, nor in predicting a later development of atopic disease [9, 11, 12].

We and others have shown that enhanced IL-13 concentrations after allergen-specific stimulation of CB cells are a predictor for the further development of atopic

dermatitis [11, 13]. Other groups have reported contrary results and found that reduced IL-13 [14, 15] or IFN- γ [16, 17] concentrations are predictors for the later development of allergic diseases. Both observations might be explained by a skewed balance of T-helper type 2 (Th2)/Th1 directed by regulatory T cells [18]. A significant limitation of all these studies is that the different methodological approaches hamper a comparison of the somewhat conflicting results. More importantly, Thornton et al. [19] challenged current interpretations of the relationship between *in vitro* T cell reactivity in human infants and the expression of allergies later in life. They reported that neonatal T cell cultures display high levels of apoptosis, and stressed the point that the neonatal T cell reactivity should be regarded as an initial burst of short-lived cellular immunity, which is regulated via parallel activation of CD4⁺CD25⁺ cells. In this respect, the interpretation of altered early T cell reactivity as a potential marker of subsequent allergic disease needs revision [19].

Therefore, the requirements for possible predictive parameters from CB are twofold. First, from a practical point of view, it is important to agree on the use of standardized tests. Second, for the evaluation of predictors, rather than T cells it might be equal or even more appropriate to review changes in the reactivity of eosinophils or basophils, which are major contributors to the allergic inflammation.

About 15 years ago, a cellular test based on the quantification of sulphidoleukotrienes (SLT C4, D4 and E4), produced and released by IL-3-primed basophils in response to allergens, was developed and became commercially available [20].

SLTs are key mediators of the systemic allergic immune responses that lead to the typical symptoms of allergic diseases, including bronchoconstriction and wheezing in asthma, increased mucus secretion, vasodilatation and increased microvascular permeability, which lead to tissue oedema and can prolong eosinophil survival [21–24]. They are produced by eosinophils, basophils, macrophages, mast cells, and to a lesser extent T cells and endothelial cells [21]. Enhanced allergen-specific SLT concentrations can be observed in atopic individuals. Therefore, SLT testing has become a complementary tool in the diagnostic algorithm of allergic disorders (reviewed by de Weck and Sanz [25]).

So far, one study has examined SLT release in neonates [26] and no data exist on the predictive value of SLT release from CB basophils.

In this study, we tested the capacity of CB cells to secrete SLTs in response to anti-IgE-receptor antibodies and to different single nutritive allergens, as well as to an allergen mix containing 21 inhalative and nutritive allergens. Furthermore, cytokine levels (IL-13 and IFN- γ) and, in a subgroup, activated CD63 basophils were quantified. The data were then compared with the parental atopy

history and the development of atopic diseases of the neonates. The aim of the study was to determine whether CB basophils, a cell type involved in the effector phase of allergic reaction, are able to release SLT in response to allergen stimulation and whether SLT levels at birth are a predictive parameter for the development of allergic diseases.

Population and methods

Participants and study design

Eighty-six samples of the CB of neonates were collected. Human umbilical CB was drawn on heparin (15 IU heparin/mL blood) before expulsion of the placenta. Method of delivery, birth weight and medical as well as family history of atopy (HA) were obtained with a questionnaire. CD 63 expression was analysed in $n = 46$ samples. Cytokine and SLT release were quantified in $n = 86$ and $n = 84$ samples, respectively. Eighteen months after birth, the cohort was reexamined. We were able to contact $n = 78$ families who received a questionnaire. Of these 78 families, $n = 70$ (90.7 %) replied.

Sulphidoleukotrienes release

CB SLT release in response to the different stimuli was measured by the CAST-Combi method (Bühlmann Laboratory AG, Allschwil, Switzerland) [27]. A total volume of 18 mL CB was centrifuged (200 g, 10 min) at 4 °C. The whole fraction of plasma and leucocytes was removed and again centrifuged (500 g, 10 min) at 4 °C. The upper fraction of plasma was discarded and the pellet was resuspended in a stimulation buffer containing 20 ng/mL IL-3. IL-3 enhances mediator release without inducing its synthesis [28]. Thereafter, aliquots of 100 μ L of the cell suspension were added in duplicate to each 100 μ L stimulation buffer as negative control, and activating monoclonal anti-IgE-receptor antibodies (Le 27, 0.5 μ g/mL; Bühlmann Laboratory AG, Schönenbuch, Germany) as positive control, and to different allergens. The allergens were supplied by Bühlmann Laboratory AG. All allergens are provided as stable, concentrated liquid forms without covalent modifications. The allergens and mixes contained 25 ng of concentrated protein extract per vial. The dilution with 250 μ L stimulation buffer results in a concentration of 100 ng/mL. For specific cell stimulation, a final concentration of 20 ng/mL allergen was achieved. The following allergen extracts were used: an allergen mixture (TOP:GX1 6-grass mix, G12 cultivated rye grass, T3 common birch, T4 hazel, W6 mugwort, W9 ribwort, M6 *Alternaria tenuis*, D1 *Dermatophagoides pteronyssinus*, D2 *Dermatophagoides farinae*, E1 cat epithelium, E2 dog epithelium, egg white (EW); PTOP: allergens of TOP+F75 egg yolk (EY), F2 milk, F3 codfish, F13 peanut, F14 soybean), BLG, α -lactalbumin (ALA), ALA, EW and EY.

After an incubation period of 40 min at 37 °C, the samples were centrifuged and then the supernatants were frozen at –20 °C until analysis of SLT levels using a murine anti-SLT monoclonal antibody-based ELISA. The anti-SLT monoclonal antibody possesses a similar affinity for LTC₄ and its two metabolites, LTD₄ and LTE₄, but does not cross react with LTB₄, arachidonic acid or other compounds with L-cysteine or with glutamine side chains. The value of the negative control was subtracted from the stimulated samples beforehand and all results were expressed as picograms per millilitre. A positive response to one of the tested stimuli was defined as (1) a ratio of stimulated to unstimulated cells >2, and (2) an absolute SLT release of >125 pg/mL above the individual baseline value.

Flowcellular allergen stimulation test

Using CellQuest software [29], flow cytometric analysis of surface markers was performed at 488 nm on a FACScan flow cytometer equipped with a 15-nW argon ion laser (Becton Dickinson, Heidelberg, Germany). On the histogram defined by forward scatter and side scatter, the initial cell gate was defined by a bit map around lymphocytes. The second gate was defined around cells showing high-density anti-IgE label or anti-CC-chemokine receptor (CCR)-3, respectively, identifying them as basophils. In the assay, a mean number of 508 basophils were assessed (5th–95th percentile 441–576). The cells were stained with anti-CD63-fluorescein-5-isothiocyanate (FITC) and anti-CCR-3-phycoerythrin (PE) or anti-IgE-PE, respectively. The isotype controls are a part of the negative control. The antibodies used in the flow cellular allergen stimulation test (CAST) (B-BAT-SR) are mouse anti-CD63-PE IgG1, Klon (CLB-gran/12) (CALTAG); goat anti-IgE-FITC (CALTAG); mouse anti-CCR-3-PE IgG2b, Klon (5E8) (Abcam).

Cytokines

Whole blood was diluted one in five with an ultra culture medium supplemented with 1% L-glutamin, penicillin-streptomycin. The diluted blood was placed in 24-well culture plates (1 mL/well). For cytokine analysis, whole blood was cultured in the medium alone or stimulated with phytohaemagglutinin (PHA, 25 µg/mL; Sigma, Taufkirchen, Germany), BLG (25 µg/mL; Sigma) and ovalbumin (OVA, 25 µg/mL; Sigma).

The supernatants of the unstimulated and the PHA-, BLG- and OVA-stimulated cells were obtained after 48 h of culture at 37 °C in humidified 5% CO₂/95% O₂. They were then analysed using commercially available ELISA technique (Coulter-Immunotech, Krefeld, Germany) in accordance with the manufacturer's instructions. All assays of the specifically stimulated CBMC and platelet-derived

Table 1. SLT release (pg/mL) in response to the medium alone (negative control = baseline) and in response to anti-Fc-IgE antibody (positive control), allergen mix TOP and PTOp, BLG, ALA, egg white and egg yolk

	SLT release (pg/mL)	SLT release above baseline (pg/mL)
Negative	124.2 50.9–333.6	
Positive	3871.5 969.6–12 563	3721.6 914.4–12 345.8
Allergen mix (TOP)	123.9 52.1–359.5	2.05 – 43.6 to 119.3
Allergen mix (PTOP)	128.5 58.5–414.9	9.5 – 41.9 to 225.1
BLG	125.2 53.4–414.1	1.0 – 50.8 to 145.4
ALA	122.3 35.2–295.1	– 3.5 – 74.5 to 134.7
EW	113.1 36.9–348.7	– 7.5 – 74.0–110.0
EY	108.4 42.8–328.2	– 5.4 – 53.1 to 69.6

The median, 5th and 95th percentile are assigned. The left column shows the absolute SLT release, the right column shows the SLT release above baseline [Δ above baseline = SLT release in response to stimulus – basal SLT release (negative control)].

SLT, sulphidoleukotriene; BLG, β -lactoglobulin; ALA, α -lactalbumin; EW, egg white; EY, egg yolk.

growth factor were performed in duplicate. The limit of detection was 1.5 pg/mL (IL-13) and 1 pg/mL (IFN- γ).

Statistical analysis

All analyses were performed using the Statistical Analysis System (SAS, Version 6.12). Differences between the groups were tested using the Wilcoxon two-sample procedure. The differences before and after treatment were analysed using the Wilcoxon-one sample procedure.

Results

A total of 86 CB samples from neonates ($n = 42$, 48.8% boys; $n = 44$, 51.2% girls) were collected. The median birth weight was 3255 g (5th–95th percentile 2485–5125 g) and the median gestational age was 39 weeks (range 37–41 weeks). Of the neonates, $n = 40$ (46.5%) had at least one atopic family member (18.6% atopic mother, 14% atopic father, 10.5% mother + father atopic, 3.5% atopic sibling).

Cellular allergen stimulation test-enzyme-linked immunosorbent assay

The absolute SLT release in response to the different stimuli is summarized in Table 1. In addition, the SLT release of stimulated blood samples is also presented as values above the unstimulated negative control. The

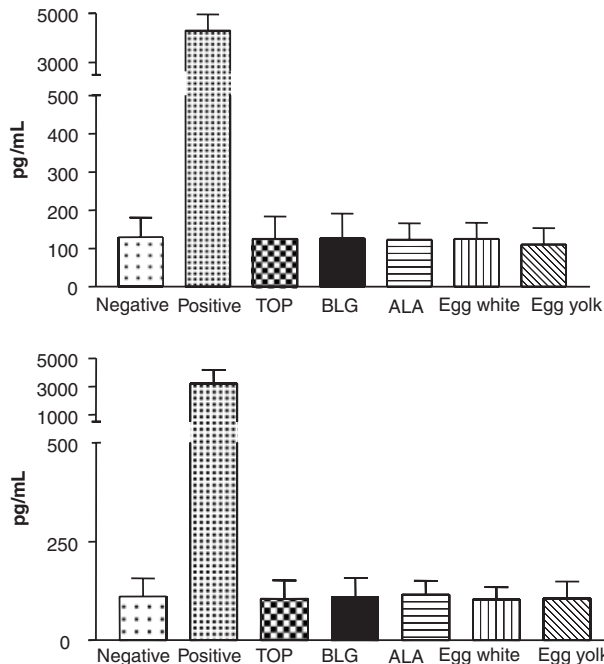


Fig. 1. Sulphidoleukotriene release of unstimulated and stimulated cord blood basophils of atopy-prone (upper part) and not atopy-prone (lower part) neonates. No significant statistical difference could be observed between these two groups (Wilcoxon test).

highest SLT release was observed in response to the unspecific stimulus anti-Fc-IgE antibody, with a median release of 3871.5 pg/mL. The median of the allergen-specific SLT release was comparable with baseline SLT release. Next, we divided the population into neonates with and without a positive HA⁺ (Fig. 1). Comparing the SLT release between these two groups, no difference could be observed in response to the positive control or any of the tested allergens. We observed no differences between atopy-prone and not atopy-prone neonates in terms of the number of basophils in CB. In addition, calculating the amount of SLT in relation to the number of basophils did not show any difference between atopy-prone and not atopy-prone neonates (data not shown).

Finally, we defined a subgroup of individuals with a specific SLT release above baseline. We defined the specific SLT release as (1) a ratio of stimulated to unstimulated cells >2, and (2) an absolute SLT release of >125 pg/mL above the individual baseline value.

Based on this definition, 98% ($n = 82$) showed a specific SLT release in response to the positive control, whereas only $n = 13$ (15.47%) responded to one of the tested allergens. Again, no difference between neonates with and without an atopic family history could be observed (Table 2).

To evaluate whether neonates with HA respond to a higher number of allergens than those from families with no HA, we considered only the reactivity to a single allergen. Excluding reactions to an allergen mix (TOP, PTOP), atopy-prone neonates responded to a 1.8 times higher number of aller-

Table 2. Cord blood samples with an allergen-specific SLT release to one of the tested allergens

	Allergen	
	Definition 1: ratio of stimulated to unstimulated cells >2	Definition 2: absolute SLT release of >125 ng about the individual baseline value
No HA		
1	TOP	TOP
2	PTOP	PTOP
3	PTOP	PTOP
4	BLG	BLG
5	ALA	ALA
6	BLG	BLG
7	PTOP	
8	EY	
9	TOP, EY	
10		PTOP
11		PTOP
12		BLG
HA		
1	PTOP, TOP	PTOP, TOP, EW
2	PTOP, TOP	TOP, PTOP
3	PTOP, BLG, ALA, EW	PTOP, BLG, ALA, EW
4	PTOP	PTOP
5	BLG, ALA, EY	PTOP, BLG, ALA, EW, EY
6	BLG	BLG
7	ALA	ALA
8	PTOP	
9	EW	
Total number of reactive individuals	$n = 18$ (21.42%) $n = 13$ (15.47%)	$n = 16$ (19.05%)

Two definitions were used: (1) a ratio of stimulated to unstimulated cells >2, and (2) an absolute SLT release of >125 ng about the individual baseline value.

SLT, sulphidoleukotriene; HA, family history of atopy; BLG, β -lactoglobulin; ALA, α -lactalbumin; EW, egg white; EY, egg yolk; TOP, PTOP, allergen mix.

gens than non-atopy-prone neonates. However, the number of individuals with an allergen-specific reaction is too low to make definitive conclusions.

Flow cytometry

In contrast to blood samples of toddlers, we were not able to detect CD63⁺ basophils upon stimulation with an anti-IgE-receptor antibody on basophils labelled with an anti-IgE antibody ($n = 40$). In contrast, CCR-3-labelled basophils showed a marked response with significant up-regulation of CD63 after stimulation with anti-Fc-IgE-receptor as well as formyl-Met-Leu-Phe (fMLP) ($n = 6$; Table 3). In this subgroup, we observed no CD63 expression in response to the tested allergens BLG and the allergen mix TOP (Fig. 2).

Cytokine concentrations

Next, we examined the concentrations of the TH2 cytokine IL-13 and the TH1 cytokine IFN- γ in the supernatants of the stimulated whole CB cultures. The highest cytokine concentrations were observed in response to the unspci-

Table 3. Percentage of the CD63 cell expression in CC-chemokine receptor-3-stained basophils in response to different stimuli of $n = 5$ cord blood samples (CB1-CB5)

	CB 1	CB 2	CB 3	CB 4	CB 5
PB	4.8	0	2.67	2.0	1.2
Anti-IgE-AK	68.1	69.9	89.7	90.1	83.8
fMLP	11.9	19.9	39.9	23.7	36.4
BLG	4.8	1.3	2.2	2.6	2.1
TOP	–	1.5	1.3	4.2	4.8

CB, cord blood; PB, phosphate buffer; anti-IgE-AK, anti-IgE receptor antibody; fMLP, formyl-Met-Leu-Phe; BLG, β -lactoblobulin; TOP, allergen mix.

fic stimulus PHA (median IL-13 concentration 591.9 pg/mL; 5th–95th percentile, 91.8–2271.5; IFN- γ 724.2 pg/mL; 29.4–8823). In response to the tested allergens, the median IL-13 release was 4.15 (BLG; 5th–95th percentile, 0.8–20.6 pg/mL) and 1.5 (OVA; 0.3–5.3 pg/mL), respectively. The median IFN- γ release was 85.1 (BLG; 5th–95th percentile, 7.8–733.3 pg/mL) and 20.1 (OVA; 2.2–117.4 pg/mL), respectively. We observed neither any differences in the cytokine release of atopy-prone and not atopy-prone neonates nor any correlation between cytokine concentrations and SLT release (data not shown).

Questionnaire

We were able to contact 78 families from our birth cohort (90.7%). Of these 78 families, $n = 70$ (81.4%) returned a completed questionnaire.

A core questionnaire for eczema was completed by the parents when the children were 18 months of age. The

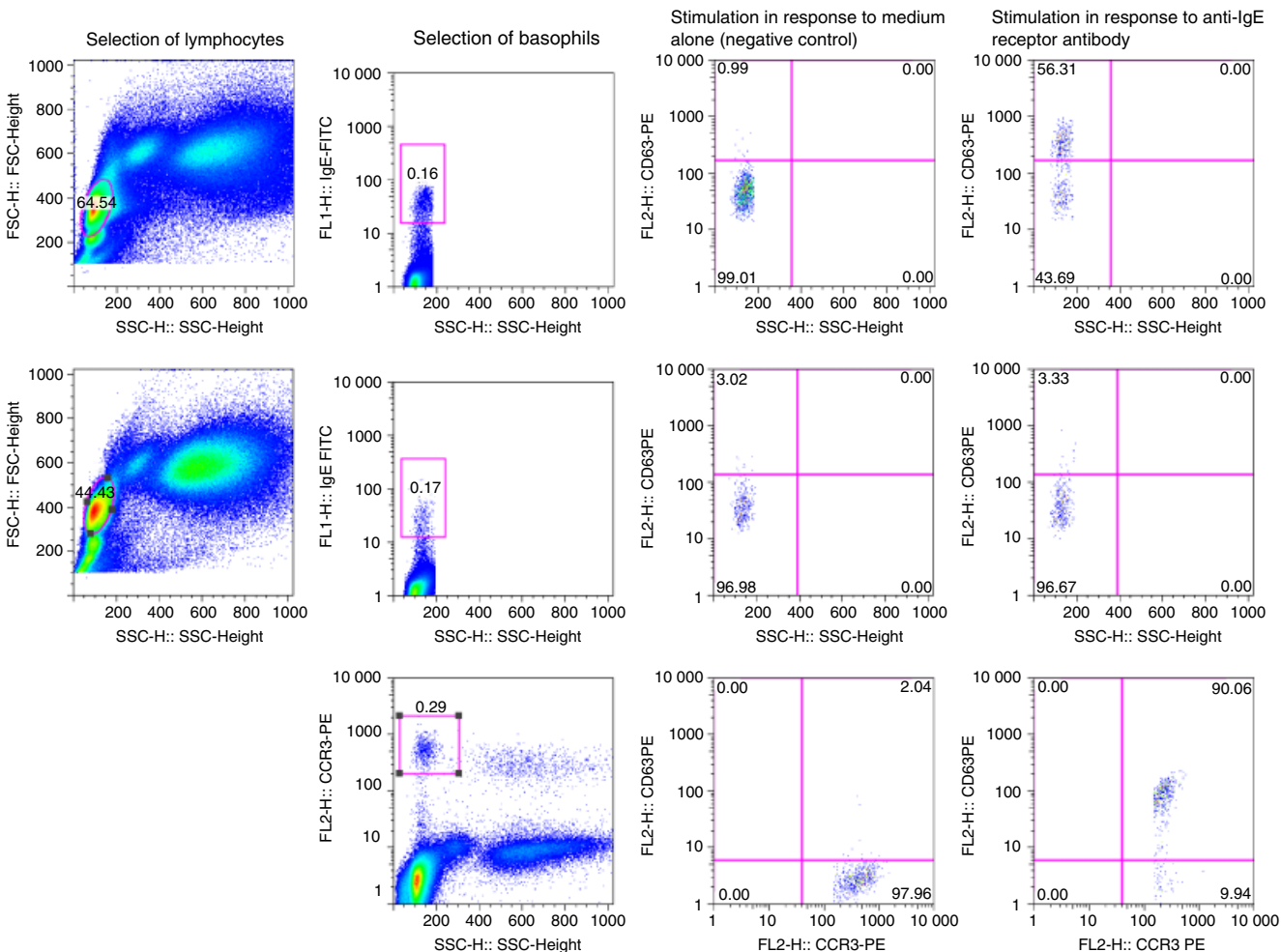


Fig. 2. Percentage of CD63⁺ cells in three representative blood samples: a 2-year-old boy (upper row) and two neonates. We showed the selection of lymphocytes and basophils, the number of CD63⁺ basophils after staining with anti-IgE and CC-chemokine receptor-3 in response to medium alone (negative control) and to anti-IgE-receptor antibody as a positive control.

Table 4. Number of children with the diagnosis of an atopic dermatitis or more than one episode of an obstructive bronchitis in relation to allergen-specific SLT release in cord blood samples

	CAST positive	CAST negative	Σ
Atopic dermatitis	1 (1.4%)	5 (7.1%)	6
No atopic dermatitis	18 (25.7%)	46 (65.7%)	64
Σ	19	51	<i>n</i> = 70
≥1 wheezing bronchitis	2 (2.9%)	8 (11.4%)	10
No wheezing bronchitis	17 (24.3%)	43 (61.4%)	60
Σ	19	51	<i>n</i> = 70

CAST, cellular allergen stimulation test; SLT, sulphidoleukotriene.

questionnaire was adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [1]. For the statistical analysis, children who were reported to have had an itchy rash on a typical place for at least 6 months were defined as 'children with symptoms suspicious of atopic dermatitis'.

All children who reported complaints suspected of being atopic dermatitis were seen by a physician (M. V. K.) to confirm or reject the diagnosis. In the end, *n* = 7 children (10%) had a definitive diagnosis of an atopic dermatitis, but only 1 of these children had an allergen-specific SLT release at birth (Table 4). Similarly, we observed no relationship between episodes of wheezing bronchitis (>1 episode; *n* = 10, 14.3%) and an allergen-specific SLT release in CB (Table 4).

Discussion

This report describes for the first time the results of a prospective study on SLT release of CB basophils in families with and without an HA for the prediction of allergic disease in childhood. We collected 86 CB samples and evaluated the symptoms of atopic dermatitis at the age of 18 months. Here, we were able to show that 15.5% of the neonates have an allergen-specific SLT release independently of their HA. Moreover, the allergen-specific SLT release at birth was not associated with a later development of atopic eczema.

The interpretation of the results of the cellular allergen stimulation test is absolutely dependent on the definition of a specific boosted SLT release. Based on published data [25], we used two definitions for a positive response to the tested stimuli: (1) a ratio of stimulated to unstimulated cells > 2 (*n* = 18; 21.4%), and (2) an absolute SLT release of > 125 pg/mL above the individual baseline value (*n* = 16; 19.5%). The value of 125 pg/mL above baseline was chosen because we observed a median SLT release of 125 pg/mL in response to medium alone in our population. For the assignment of a stimulus-specific SLT release, both criteria must be fulfilled (*n* = 13; 15.5%).

With respect to this definition, almost all CB basophils showed a positive response to the unspecific stimulus anti-Fc-IgE antibody (98%). In accordance with our result,

a percentage of 3–5% non-releasers in response to anti-IgE-receptor antibody is described in the literature [27, 30]. Until now, there has been only 1 study that has examined SLT release of CB basophils. In contrast to our findings, Szépfalusi et al. [26] reported that only 21 of 86 (20%) CB samples responded to the unspecific stimulus anti-IgE. A different definition of stimulus-specific SLT release and different protocols might explain the discrepancy between the results of Szépfalusi et al. and our findings. Szépfalusi et al. defined SLT values >200 pg/mL over background as a positive response. However, with respect to our positive control, the number of responders remained unchanged (98%) using exactly the same definition.

Therefore, methodological difference might be crucial. We included only CB samples of neonates after caesarean section to guarantee immediate transport to our laboratory and immediate laboratory work-up. The efficient handling might be associated with a higher number of viable cells in our assays, which are more reactive to produce SLT. Moreover, Szépfalusi et al. [26] diluted the CB at a 1 : 1 ratio with physiological phosphate-buffered saline, while we used undiluted blood samples. We speculate that the dilution of CB cells might also affect either the viability of basophils or the ability to produce and release SLT, or both. Finally, Szépfalusi et al. used an anti-IgE antibody as a positive control, while we used an anti-IgE-receptor antibody. Based on data of the literature, the percentage of non-releasers in response to anti-IgE-receptor antibody is slightly higher (about 10%) [31, 32] than non-releasers in response to anti-IgE antibody. Moreover, based on our data obtained from the flow cytometry analysis, we speculate that neonates have a lower number of IgE-positive basophils than toddlers. This might additionally explain the different results compared with the data of Szépfalusi et al. They studied only the allergen-specific response to an allergen mix and observed an allergen-specific SLT release in 24 of 104 (23%) CB samples, which is comparable to our findings.

One weakness of our study is the fact that we used only one concentration of the tested allergens. In terms of segregation between negative and positive responses, a single allergen dose of 10–20 ng protein is recommended and believed to be sufficient, because the dose–response

curve has a broad bell shape over three to four potencies [33]. Nevertheless, a protocol with titrated allergen concentrations for basophil stimulation might be a more sensitive approach to reveal an allergen-specific SLT release. Our currently used protocol could result in a slightly underestimated proportion of neonates with allergen-specific SLT release. Moreover, it might be worth discussing the combination of allergen extracts used in our study. It has to be annotated that the allergen mix PTOP contains a number of nutritive allergens, while some relevant allergens such as casein are missing. This might also result in slightly underestimating the neonatal allergen-specific SLT release.

A combination of the determination of SLT release, which can be quantified in the CAST, and basophilic activation by flow cytometry is of diagnostic value for drug and latex allergy [29, 34, 35]. Therefore, we also aimed to quantify the number of CD63⁺ basophils in response to different stimuli in CB. In contrast to basophils from toddlers, we detected no CD63⁺ basophils upon stimulation with an anti-IgE-receptor antibody on basophils labelled with an anti-IgE-antibody. As discussed above, we speculate that probably because of low levels of IgE in CB, an anti-IgE receptor is not applicable for basophil staining. In contrast to anti-IgE, staining with the human eotaxin-receptor CCR-3 revealed a population of basophils with marked response after stimulation with fMLP, which is a synthetic peptide that mimics the activity of bacterially derived peptides with formylated N-terminal methionine groups. Interestingly, CCR-3-positive basophils in CB also respond after stimulation of the IgE receptor, indicating that basophils express a sufficient density of FcεR1.

Besides CD63⁺, basophils upregulate the expression of different markers such as CD45⁺, CD203c⁺ or CD63⁺ upon stimulation (reviewed by Ebo et al. [36]) Because quantification of CD63⁺ on activated basophils is a well-established setting and the superiority of other markers is not clearly shown, we used only CD63⁺ to quantify basophil activation.

We observed no difference between SLT release of neonates with and without an HA. This criterion was fulfilled when either the father or mother or one sibling had an atopic eczema, asthma or AR. When comparing neonates with an atopic mother alone (or with an atopic father alone) and neonates without an HA, this result was unchanged. Therefore, the ability of CB basophils to produce SLT is independent of HA. Moreover, the ability of CB basophils to produce SLT is not associated with an increased risk for atopic eczema at an age of 18 months. From seven children with an atopic eczema, only one had an allergen-specific SLT release at birth. On the other hand, one child with a confirmed food allergy to chickens' eggs showed no allergen-specific SLT release at birth.

The clinical outcome of this study 'atopic symptoms' was obtained by means of a questionnaire using the ISAAC items [1]. All children who reported complaints suspected of being atopic dermatitis were seen by a physician (M. V. K.) to confirm or reject the diagnosis. Even if it were desirable to collect more objective data, i.e. blood sampling and IgE testing, we abstained from invasive procedures for reasons of feasibility. However, despite some limitations based on our study design, we believe that the information obtained by the ISAAC questionnaire and the clinical examination can be used as a surrogate for the clinical objectivity in children with atopic disease.

Taking these results together, SLT release at birth does not seem to be a useful informative parameter for the development of atopic eczema.

The cellular allergen stimulation test is a suitable approach to diagnose allergic diseases. In general, in comparison with the skin prick test (SPT) or specific IgE determination, the CAST is not more sensitive in children [37]. Nevertheless, in individual cases, where the SPT is difficult to perform and specific IgE testing is not decisive, the CAST might be a complementary tool for the definitive diagnosis of allergy. However, the quantification of CB basophils SLT in response to allergens is not a useful approach for detecting children at risk for the development of atopic eczema.

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