

Short communications

Neonatal leukocyte cAMP-phosphodiesterase determination is not suitable for allergy prediction

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The atopy-predictive value of cord blood (CB) cyclic adenosine monophosphate-phosphodiesterase (cAMP-PDE) in mononuclear leukocytes (MN) was studied prospectively in 57 neonates. Reliable determination of cAMP-PDE was achieved in only 20 CB samples. The levels tended to be higher in infants developing signs of atopy up to 18 months of age. The combination of CB cAMP-PDE and family history (FH) of allergy showed a higher predictive value ($P = 0.040$) than each of them separately ($P = 0.370$ and $P = 0.102$, respectively). The cAMP-PDE levels in CB also correlated to FH of allergy/atopy in accordance with earlier studies ($P = 0.027$). In conclusion, determination of cAMP-PDE in neonates may have some predictive value, but it is not suitable for general screening.

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The presence of elevated umbilical cord blood (CB) IgE levels and a family history (FH) of atopic/allergic diseases is associated with later allergic manifestations (1, 2). The predictive value of several other tests in early infancy has also been assessed, including eosinophil and platelet counts in peripheral blood; skin histamine reactivity; basophil histamine releasability; and IgG, IgE, and IgD antibodies in amniotic fluid and CB. However, none of the applied methods are satisfactory for clinical use, mainly because of poor sensitivity.

Elevated cyclic adenosine monophosphate-phosphodiesterase (cAMP-PDE) activity in CB mononuclear leukocytes (MN) has been observed in infants of atopic parents (4). We therefore decided to study, prospectively from birth, the predictive value of determination of cAMP-PDE activity in CB MNs with regard to manifestations of atopic disease up to 18 months of age.

Material and methods

The study group comprised 57 neonates (in Motala, Sweden) with known family history of allergy. CB

samples were collected in heparinized (10 U/ml) test tubes after thorough cleaning of the cord with a dry compress. MNs were isolated, within 24 h, on Hypaque-Ficoll (Pharmacia, Uppsala, Sweden) gradients, according to Heskell et al. (4) with certain modifications. Thus, before separation, CB was diluted 1:3 with Hanks' balanced salt solution (Flow Svenska, Solna, Sweden). Contaminating erythrocytes in the monocyte layer were lysed by hypotonic shock, with 6 ml water and 2 ml phosphate buffer for 20 s. The cells were then washed twice and resuspended in Gey's buffer. Cell samples were stained with Wright's stain, counted in a Bürker chamber, stored at -70°C , and then transported in frozen condition to Portland, OR, USA, for analysis.

The cAMP-PDE levels were measured in cell samples after freeze-thawing three times by a modification of the procedure of Thompson et al. (10). The cAMP-PDE assay incubation mixture (0.4 ml) contained 0.02 mM cAMP, 20 000 cpm ^3H -labeled cAMP, and a 0.02-ml sample of 3.75 mM β -mercaptoethanol and 50 mM MgCl_2 in 40 mM TRIS-HCl buffer (pH 8.0). After incubation (30°C for 10 min), the reaction was terminated by snap-freezing in

ethanol/dry ice, and the mixture was boiled for 1 min. Purified 5'-nucleotidase (0.45 U) was then added, and the samples were incubated at 30°C for 10 min and transferred to Pasteur pipette columns containing AGI-X2 resin. Eluates were collected in scintillation fluid, and ³H-labeled adenosine was quantitated in a Packard Tri-Carb scintillation spectrometer. Enzyme activity was expressed as picomoles of cAMP hydrolyzed per min/mg protein (4).

The MN samples were analyzed for cAMP-PDE content by two of the authors (SC, JH) without their knowing the atopic state of the children or of their family members.

Despite substantial effort, acceptable cell separation was achieved in only 20 CB samples, allowing cAMP-PDE analyses of MNs without disturbing contamination of erythrocytes, neutrophils, eosinophils, and immature forms (9).

Serum IgE was determined with a modified version of Phadebas IgE PRIST® (Pharmacia, Uppsala, Sweden), permitting determination of low concentrations (3). For detection of any maternal contamination of the cord serum, radioallergosorbent tests (Phadebas RAST®, Pharmacia, Uppsala, Sweden) were performed on cord sera when the IgE levels exceeded or were equal to 0.5 kU/l with allergens against which the mother was skin-prick-test (SPT) positive, i.e., birch, timothy, cat, and dog. All these tests on cord blood were negative.

At 3, 6, 12, and 18 months of age, the parents completed questionnaires on allergic symptoms and signs in their babies. They were also offered a consultation and treatment if needed. A physical examination and SPT with a panel of nine allergens were done at 18 months. The SPTs were done in duplicates as described elsewhere (8) with birch pollen, *Dermatophagoides farinae*, *D. pteronyssinus* (Phazet®, Pharmacia, Uppsala, Sweden), cow's milk, hen's egg, wheat, and rye (Dome/Hollister-Stier/Bayer, 1:20 w/v). If the family had a pet animal, the corresponding Phazet allergen was added to the panel. A mean diameter equal to, or greater than, that of Phazet Histamine on the same occasion, and/or equal to, or greater than, 3 mm was regarded as positive. A venous blood sample was obtained after topical administration of anesthetic ointment (EMLA®, Astra, Södertälje, Sweden). The parents also completed a questionnaire on symptoms of allergic disease in the immediate family. A history of allergy among siblings was confirmed by case records. The information was also verbally confirmed during the visit to the doctor. In addition, venous blood samples were obtained from both parents. The atopic state of the parents was assessed by SPT with 11 common allergens (see below).

The atopic state of each child was determined from clinical findings and the questionnaire infor-

mation. Kjellman & Johansson's clinical classification was used with minor modifications to suit 18-month-old children (7). The children were classified as either having or not having atopic disease.

For classification of FH, the previously described family allergy score (FAS) was used (6). Obvious atopic disease in the mother, father, or a sibling was scored 2 points, and 1 point was scored for probable disease. Only the highest point of a sibling was added to the parents' FAS. Thus, the maximum FAS was 6 points.

All the mothers and 15 fathers were tested with a panel of 11 Phazet allergens: birch, timothy, ryegrass, mugwort, horse, dog, cat, *Cladosporium*, *Alternaria*, *D. farinae*, and *D. pteronyssinus*, together with histamine and a negative control.

The cAMP-PDE levels in the atopic and non-atopic groups at birth and at 18 months of age were compared by the Mann-Whitney U rank sum test and simple regression analysis. The chi-square test with continuity correction was used in four-field table situations, and the Mantel-Haenszel chi-square test was used when data consisted of several four-field tables (5).

The study was approved by the Human Research Ethics Committee at the University Hospital, Linköping, Sweden. Informed consent was given by the parents before entering the study.

Results and Discussion

The median and mean MN cAMP-PDE levels (picomoles of cAMP hydrolyzed per min/mg protein) were 99.80 and 106.39 in the nonatopic ($n = 14$) and 121.50 and 127.43, respectively, in the atopic children ($n = 6$; $P = 0.409$) (Fig. 1, panel A). The CB cAMP-PDE levels in the three most prominently atopic children were 95, 99, and 145 pmol/min per mg protein, as compared with 25, 29, 135, and 203 in the four most convincingly nonatopic children. Thus, the cAMP-PDE levels at birth did not predict later atopic disease.

The CB MN cAMP-PDE content was higher in infants with FH of allergy ($P = 0.027$; $n = 20$) (Fig. 1, panel B). This agrees with previous findings (4).

Neither CB IgE ($r = 0.330$; $P = 0.624$; $n = 17$) nor IgE at 18 months ($r = 0.181$; $P = 0.536$; $n = 14$) showed any significant correlation with CB MN cAMP-PDE. As shown in previous studies (7), the IgE levels in CB and at 18 months correlated closely ($r = 0.796$; $P = 0.003$).

With the Mantel-Haenszel chi-square test, FAS and CB cAMP-PDE were studied in combination as predictors of later atopy. With cutoff levels for FAS at 3 score points and for CB MN cAMP-PDE at 90 pmol of cAMP hydrolyzed per min/mg protein, a significant difference appeared in this test between

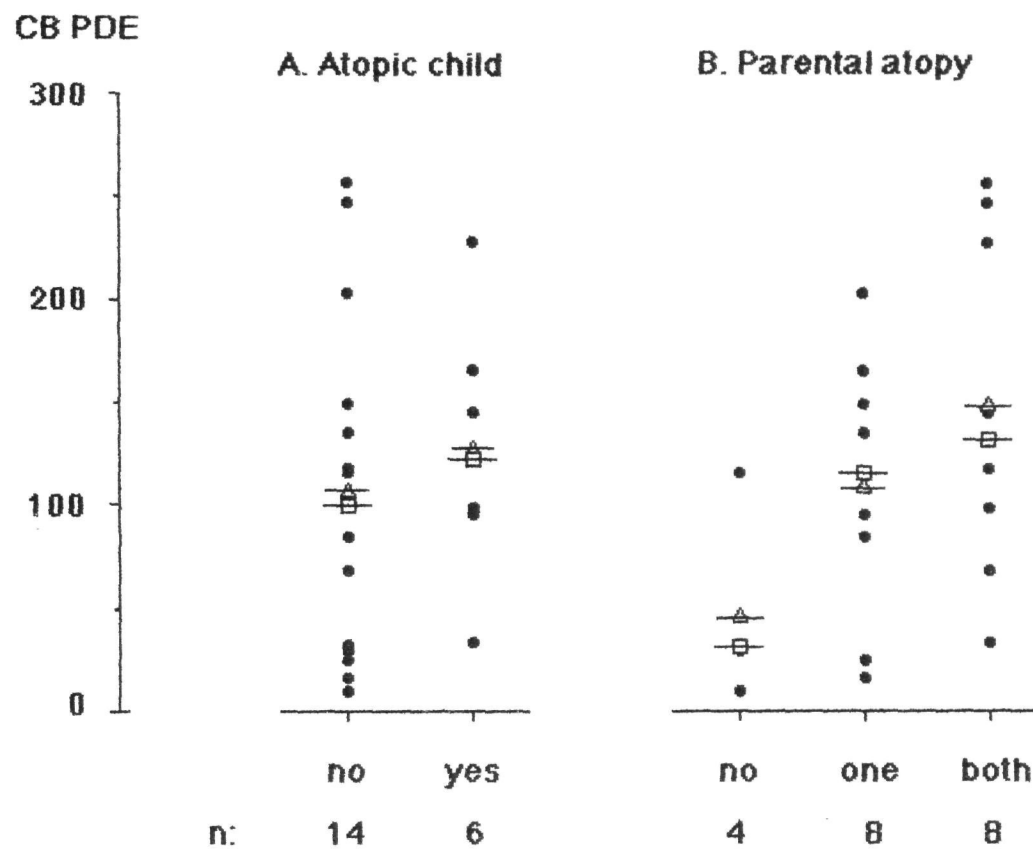


Fig. 1. Cord blood mononuclear leukocyte content of cAMP-phosphodiesterase (CB PDE) in pmol cAMP hydrolyzed per min/mg protein in 20 samples in relation to (A) development of atopic manifestations in children up to 18 months of age, and (B) parental atopic manifestations. Median values are indicated by squares and means by triangles.

nonatopic and atopic children ($P = 0.040$). Neither FAS nor CB cAMP-PDE separately predicted atopy at significant levels ($P = 0.370$ and $P = 0.102$, respectively).

Although the study population was small, it seems appropriate to conclude that determination of cAMP-PDE does not appear to be highly predictive of later allergic disease. As the method is relatively complicated and demands a highly separated cell population, it does not seem to be a satisfactory screening test.

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