

ORIGINAL ARTICLE

Oxidative stress status in umbilical cord blood from neonates born to mothers with atopic asthma

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Abstract

Objective: Our objective was to assess the oxidative stress status and analyse the relationship between an oxidant/antioxidant imbalance and the mediator release properties of cord blood basophils from neonates born to mothers with atopic asthma.

Methods: Cord blood was collected from the neonates of 16 asthmatic mothers and 18 healthy mothers. Basophils were purified and stimulated by *Dermatophagoides farinae* (Df), hyperosmotic mannitol or peptidoglycan (PGN). Immunoblotting detected nuclear factor κ B (NF- κ B) as a measure of functional receptor response. The linear correlations between IL-4 levels in the supernatants and 3-nitrotyrosine (3-NT), glutathione peroxidase (GSH-Px) in the serum were evaluated.

Results: Compared with the healthy group, the levels of 3-NT in maternal blood and cord blood were significantly higher in allergic asthma group, whereas the GSH-Px activity were lower. After stimulation, cord blood basophils from the neonates born to atopic mothers produced more IL-4 involving NF- κ B pathways. There was a significant relationship between the IL-4 levels produced by basophils and 3-NT (or GSH-Px) in cord blood from allergic asthma group.

Conclusion: In asthma, mediator release properties of human basophils induced by environmental allergens and irritants are associated with oxidative stress, which may be one of the pathogenesis of allergic diseases.

Keywords

Allergic asthma, basophil, interleukin-4, oxidative stress, umbilical cord blood

History

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Introduction

Since chronic airway inflammation is a striking feature of asthma, asthmatic airway inflammation is often assumed to be the initiating event for airway remodeling [1]. Inflammation in the airways increases the risk of oxidative damage. A large body of data suggests that free radical oxidative damage – particularly of lipids, nucleic acids and proteins – is extensive in the lungs of ovalbumin (OVA)-sensitized asthmatic mice [2,3]. In the pathogenesis of asthma, oxidative stress appears to play an important role and existence of an oxidant/antioxidant imbalance is evident. 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children [4]. Lower GSH-Px activity in children with controlled asthma showed deficient erythrocyte antioxidant defence and evidence of association between oxidative stress and asthma in childhood [5].

It has been widely reported that basophils readily secrete Th2 cytokines (e.g. IL-4), which provides new insights into possible roles basophils may play in atopic disease progression and/or immune responses [6,7]. Some data support a primary role for basophils in the recruitment of additional inflammatory cells rather than as effectors of IgE-mediated chronic allergic inflammation (IgE-CAI) [6] since basophils are primary sources of IL-4 production [8] and activated basophils release IL-4 earlier than T cells driving Th0 to Th2 differentiation [9].

Several lines of evidence support the concept of fetal programming and very early life events in the development of the different phenotypes of asthma [10,11]. The mechanisms that affect fetal development in pregnancies complicated by asthma are not clearly defined. The current study was designed to characterize the profile of 3-NT and GSH-Px in cord blood of neonates born to mothers with allergic asthma and IL-4 production by human basophils following several stimuli challenges to determine whether oxidative stress was associated with the mediator release properties of human basophils as a means of promoting the differentiation of Th0 to Th2 and development of allergic diseases.

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Materials and methods

Reagents

The skin prick test kit was purchased from Allergopharma (Allergopharma, Reinbek, Germany). CD45-PerCP (peridinin chlorophyll protein complex) and CD203c-PE (phycoerythrin) were obtained from BD Biosciences (San Jose, CA) and Milteny Biotec (Bergisch Gladbach, Germany). Human 3-NT, GSH-Px and IL-4 ELISA Kits were purchased from Abway Antibody Technology Co Ltd (Beijing, China), Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and Bender MedSystems (Vienna, Austria) respectively. PGN was purchased from Sigma (Buchs, Switzerland). Monoclonal NF κ B (p65 subunit) antibody was purchased from BdBioscience Transduction Laboratories (San Diego, CA).

Subjects

Atopic patients

Sixteen asthmatic mothers (average age 29 ± 2 years, 39 ± 2 weeks of gestation), whose asthma symptoms were declining, were enrolled in this study. Asthma diagnosis was consistent with parameters established by the 2006 Guidance for Branchial Asthma [12]. Medical history and skin tests showed respiratory sensitization (allergic response to *Dermatophagoides farinae* [Df]). Enrolled patients did not suffer an acute episode nor did they receive systemic corticosteroids, immunotherapy, H1-antagonists or mast cell stabilizers during pregnancy. The children's biologic fathers did not have a history of atopic disease.

Non-atopic controls

Eighteen non-atopic, post partum mothers (average age 30 ± 2 years, 39 ± 1 weeks of gestation) were enrolled in the study. Mothers and the newborns' respective fathers did not have a family history of asthma, atopic disorder or other chronic diseases. No complications were reported during pregnancy. Placental cord blood was obtained from controls as well as atopic mothers.

After having received informed consent and after approval from the Ethical Review Board at the Xinhua Hospital affiliated to the Shanghai Jiaotong University School of Medicine, 60–100 ml placental cord blood and 3 ml peripheral venous blood were collected from enrolled mothers.

Study design

3-NT and GSH-Px assays

Both cord blood from neonates and peripheral blood from mothers were subjected to determination of serum 3-NT and GSH-Px levels using ELISA technique.

Basophil purification

Cord blood basophils were purified on Ficoll Hypaque and then based on CD203c⁺CD45^{int+} staining by flow cytometric cell sorting (BD Pharmingen, San Diego, CA) [13]. This protocol resulted in both high purity ($93.08 \pm 5.01\%$) and a recovery ($60.93 \pm 5.86\%$) of the cord blood basophils.

Culture conditions

Purified umbilical cord blood-derived (UCB) basophils were washed with HEPES-ACM buffer and equilibrated at 37°C , 5% CO₂ for 15 min. The cells were then incubated with PGN, Df, 9% hyperosmotic mannitol or ACM buffer for an additional 8 h as above. Stimulation was stopped by adding 10 μ l of 10 mM EDTA at RT for 5 min.

Assessment of NF- κ B-p65 nuclear translocation by Western blot analysis

Cells were harvested. Nuclear extracts were then prepared by using a commercially available kit (Active Motif, Carlsbad, CA) and were diluted with an equal volume of SDS Tris-glycine sample buffer (Invitrogen) containing 10% 2-mercaptoethanol and 1X protease inhibitors (BD Pharmingen, San Diego, CA). Electrophoresis, protein transfer, and immunoblotting using a monoclonal NF κ B (p65 subunit) antibody were performed following normal protocol. Horseradish peroxidase-conjugated antirat and antimouse secondary antibodies were used for visualization with enhanced chemiluminescence.

IL-4 assays

IL-4 levels in cell culture supernatants were quantified using a Human high sensitivity IL-4 ELISA kit.

Statistics

Student *t* test, ANOVA and Pearson correlation analyses were carried out using SPSS 17.0 software to assess statistically significant differences between the treatment groups.

Result

3-NT and GSH-Px Levels

Compared with the healthy group, the levels of 3-NT in maternal blood and cord blood were significantly higher in allergic asthma group ($p < 0.01$), whereas the GSH-Px activity were lower ($p < 0.05$) (Table 1).

PGN, Df or 9%hyperosmotic mannitol induced nuclear localization of NF κ B in basophils

Figure 1 shows a representative experiment in which the nuclear extracts of basophils in UCB from neonates born to mothers with atopic asthma treated with PGN, Df or 9%hyperosmotic mannitol contain immunoreactivity for the p65 subunit of NF κ B, as assessed by Western blot analysis. In contrast, cells treated in this manner in healthy group showed much less of the p65 subunit and were no different from cells treated in medium alone.

IL-4 production following PGN, Df and 9%hyperosmotic mannitol stimulation

Following PGN, Df and 9%hyperosmotic mannitol stimulation, the IL-4 levels produced by cord blood basophils from neonates born to atopic mothers were significantly elevated compared to the levels detected for basophils isolated from neonates born to non-atopic mothers ($p < 0.01$) (Table 2).

Table 1. 3-NT and GSH-Px levels in cord blood from neonates and peripheral blood from mothers.

	Asthma group		Healthy group	
	Peripheral blood	Cord blood	Peripheral blood	Cord blood
3-NT (μg/ml)	443.86 ± 95.2**	474.69 ± 92.55**	288.17 ± 57.23	273.7 ± 68.09
GSH-Px (U/L)	158.14 ± 34.05*	144.83 ± 46.13*	201.86 ± 52.41	190.62 ± 33.37

The data are expressed as the mean percent ± SD of observations per group.

** $p < 0.01$; * $p < 0.05$ versus the healthy group using Student's t test.

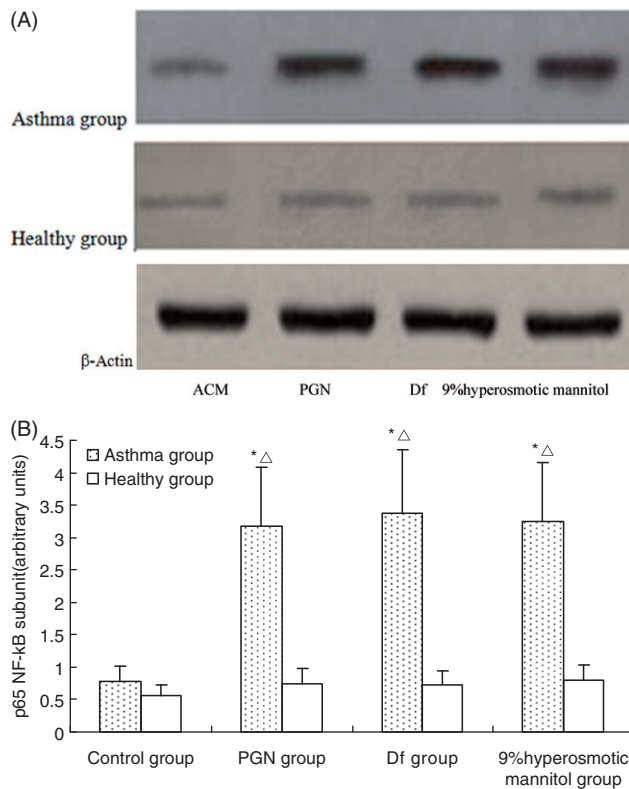


Figure 1. NF κ B (p65 subunit) expressed in nuclear extracts of basophils treated with ACM buffer alone (control group), PGN, Df or 9%hyperosmotic mannitol. Quantification and representative photograph of Western blot analysis. β -actin was used as a loading control. Representative blots are shown in (A). (B) Quantitation of results. Data are expressed as arbitrary densitometric units and are the mean $\pm$ standard deviation from each subject. * $p < 0.01$ compared with healthy group; $\Delta p < 0.01$ compared with control group.

Table 2. IL-4 protein secretion by basophils after incubation with Df, PGN or 9%hyperosmotic mannitol.

	Control group	PGN group	Df group	9%hyperosmotic mannitol group
Asthma group	1.79 ± 0.42	3.36 ± 0.39 ^{a,b}	3.27 ± 0.40 ^{a,b}	3.56 ± 0.47 ^{a,b}
Healthy group	1.65 ± 0.37	1.85 ± 0.50	1.78 ± 0.48	1.92 ± 0.52

Data are expressed as mean percent $\pm$ SD of observations per group (pg/ml).

^a $p < 0.01$ versus other conditions in each group using ANOVA analysis.

^b $p < 0.01$ versus the healthy group using Student's t test.

Correlation analysis

There was a significant positive correlation between the IL-4 levels produced by basophils and 3-NT in cord blood from

allergic asthma group following stimulation with PGN ($r = 0.621$, $p < 0.05$, Figure 2A), Df ($r = 0.607$, $p < 0.05$, Figure 2B) or 9%hyperosmotic mannitol ($r = 0.616$, $p < 0.05$, Figure 2C).

A significant negative correlation was observed between the IL-4 levels produced by basophils and GSH-PX in cord blood from allergic asthma group following stimulation with PGN ($r = -0.516$, $p < 0.05$, Figure 2D), Df ($r = -0.597$, $p < 0.05$, Figure 2E) or 9%hyperosmotic mannitol ($r = -0.508$, $p < 0.05$, Figure 2F).

Discussion

The role of genetic and environmental factors in the pathogenesis of allergic airway inflammation is complex and has not yet been elucidated [14]. For a disease beginning mostly early in infancy, it appears necessary to first understand the cellular and molecular mechanisms underlying the induction and expression of allergic reactions [15].

The prenatal environment must be regarded as a possible early risk factor for allergic diseases in children. A line of evidence now supports the hypothesis that fetal T cells are exposed during gestation to maternally derived allergens. These allergens could be ingested or inhaled by the mother [16]. Szepefalusi et al. have shown that allergen present in the maternal blood circulation (as present in the perfusion medium) is readily detected in the fetal blood circulation, providing direct evidence for transplacental maternofetal allergen transfer [17]. A larger prenatal exposure to house dust mite allergens was related to higher total IgE levels directly after birth, indicating that allergen exposure in the prenatal period can influence the fetal immune response [18]. It has been suggested that hyperosmolarity may be one of the stimuli that provoke exercise-induced asthma and rhinitis [19]. Children had a higher risk of asthma if their mothers experienced infections during pregnancy [11]. Maternal environmental characteristics, as they relate to the fetus, have also been strongly associated with subsequent asthma development [11]. Our experimental results may expound the mechanism. In our study, exposure to PGN, Df or 9%hyperosmotic mannitol could activate cord blood basophils to elevate NF κ B expression and release IL-4. Cytokines and other factors triggered as part of inflammatory processes activate NF κ B via several signaling pathways, leading to a signaling cascade that amplifies the inflammation [20,21]. Several studies have associated NF κ B overactivation with airway remodeling [20–22]. NF κ B is a critical transcription factor for Th2 cell differentiation [23]. NF κ B participate directly in the transcription of the IL-4 gene upon T cell activation [24]. NF κ B can mediate an inflammatory response

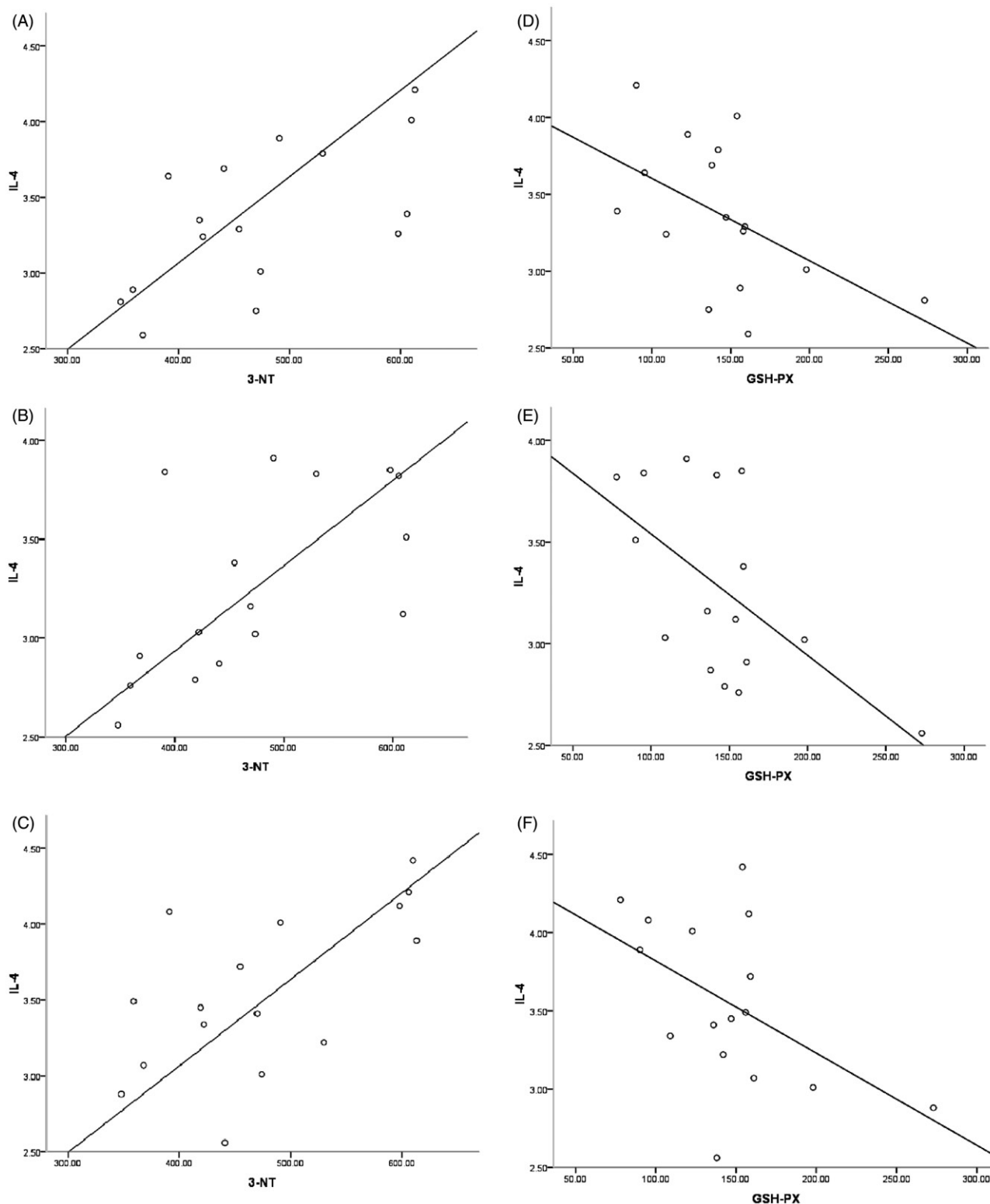


Figure 2. Correlation Analysis. Cord blood basophils from 16 neonates born to asthmatic mothers were stimulated with PGN (A, D), Df(B, E) or 9% hyperosmotic mannitol (C, F), and the IL-4 supernatant levels were positively correlated to the 3-NT (A, B, C), and negatively correlated to the GSH-Px (D, E, F). Each point on the graph represents the IL-4/3-NT or IL-4/GSH-Px result for an individual patient.

due to oxidative stress by inducing gene transcription of cytokines such as IL-1, IL-6 and TNF- α [25]. NF κ B gene and protein expressions contribute to enhance oxidative stress [26]. And, reactive oxygen species (ROS) also act as a common second messenger following cellular exposure to agents that induce NF κ B activation [27]. Thus, activation of

this transcription factor allows the establishment of a signal amplification loop.

In the pathogenesis of asthma, oxidative stress appears to play a pivotal role and existence of an oxidant/antioxidant imbalance is evident. Oxidative and nitrosative events have a crucial role in asthmatic lung disease [28]. Excessive

exposure to reactive oxygen and nitrogen species is the hallmark of oxidative stress and leads to damage of proteins, lipids and DNA [29]. In bronchial asthma, oxidative stress aggravates airway inflammation by inducing diverse proinflammatory mediators, enhancing bronchial hyperresponsiveness, stimulating bronchospasm and increasing mucin secretion [30]. Baraldi et al. found that 3-NT formation is greater in the EBC of asthmatic children, indicating that nitrosative processes are more active in the lung of asthma [31]. GSH-Px is endogenous antioxidant enzymes that serve to neutralize oxygen free radicals and hydrogen peroxide, thereby helping to control oxidative damage [32].

This is the first published study to directly investigate the oxidative stress status in UCB from neonates born to mothers with atopic asthma. In our study, compared with the healthy group, higher 3-nitrotyrosine and Lower GSH-Px levels in the serum of UCB from neonates of the mothers with allergic asthma showed that a delicate oxidant/antioxidant imbalance existed in the fetuses and newborns born to mothers with atopic asthma.

The result of correlation analysis show mediator release properties of human basophils induced by specific allergen, hyperosmolar state or some microbial components are associated with oxidative stress, which might influence developing immune responses on cord blood and clinical outcome in early life. Control of environmental allergens and irritants is essential for pregnant women to prevent their newborns from asthma.

The next study will focus on the role of antioxidants in the asthmatic pregnant women. The link between asthmatic pregnant women and their newborns in oxidative stress status will be the direction of future research and may require new methods to correct the oxidative imbalance in allergic diseases which might be one of the important methods to control the increased morbidity of allergic asthma.

Declaration of interest

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The authors report no declarations of interest.

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