

PREGNANCY

The Effect of Peptidoglycan Stimulation on Basophil-Mediated Atopic Responses During Pregnancy and in Newborns

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Background. Toll-like receptor (TLR) molecules play critical roles in directing the course of atopic diseases by recognizing specific microbial products that activate immune effector cell function. **Objective.** We determined if basophils harvested from neonates genetically predisposed to atopic disease had different levels of TLR2 expression and determined whether putative TLR2 ligands mediated cytokine secretion. **Methods.** Blood samples were collected from 10 asthmatic and 12 healthy women and their newborns. Basophil histamine was measured using the human basophil degranulation test and TLR2 expression was determined using nucleic acid hybridization *in situ* and flow cytometry. IL-4 levels were quantified by ELISA following allergen stimulation. **Results.** The basophil degranulation index (DI) in granulocytes harvested from peripheral blood of asthmatic women was assessed following stimulation with either peptidoglycan (PGN) or *Dermatophagoides farinae* (Df) extract. The DI was significantly higher in atopic women than in healthy controls. Basophils purified from the cord blood of neonates born to atopic mothers produced more IL-4 compared with basophils purified from children born to nonatopic controls. Finally, TLR2 expression at the protein and mRNA levels was upregulated in cord blood basophils from neonates born to mothers with asthma following stimulation with PGN but not Df. **Conclusion.** These data suggested that TLR2-mediated innate immune responses play a role in augmenting allergic reactions through the modulation of basophil cytokine secretion and histamine release. Microbial components may activate basophils through TLR2 (especially for genetically predisposed infants) to release cytokines associated with an increased incidence of allergic diseases.

Keywords allergen, asthma, atopic disease, basophil, IL-4, innate immunity, peptidoglycan, Toll-like receptor-2 (TLR2)

INTRODUCTION

Recently, basophil-mediated processes and signaling pathways have been utilized as indicators of allergic inflammation because the basophils serve as primary allergic-triggering cells. It has been widely reported that basophils readily produce Th2 cytokines (e.g., IL-4), which provides new insights into possible roles basophils may play in atopic disease progression and/or immune responses (1–4). 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 (2) because basophils are primary sources of IL-4 production (5, 6), and activated basophils release IL-4 earlier than T cells driving Th0 to Th2 differentiation (7).

Human basophils were recently found to express both Toll-like receptor-2 (TLR2) and TLR4 (8). Bieneman et al. (9) found that basophils selectively responded to peptidoglycan (PGN), a TLR2 ligand, and not the TLR4

ligand Lipopolysaccharides (LPS). This study focused on determining whether umbilical cord blood-derived basophils could respond to PGN similar to peripheral blood basophils as a means of determining the effects of genetic predisposition to allergic responses. This study also determined whether basophils from asthmatic postpartum women and their newborns presented with elevated TLR2 levels and whether putative ligands to this receptor affected histamine and cytokine production.

MATERIALS AND METHODS

Reagents

Dextran and Ficoll were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). CD45-PerCP (peridinin chlorophyll protein complex) and TLR2-FITC (fluorescein isothiocyanate) were obtained from BD Biosciences (San Jose, CA, USA). CD203c-PE (phycoerythrin) was obtained from Milteny Biotec (Bergisch Gladbach, Germany). IL-4 levels were determined using an ELISA kit (human high sensitivity IL-4 ELISA kit, Bender MedSystems, Vienna, Austria). The skin prick test kit was purchased from Allergopharma (Allergopharma,

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Reinbek, Germany). PGN was purchased from Sigma (Buchs, Switzerland) and TLR2 from Boster (Wuhan, China).

Subjects

Atopic Patients. Ten asthmatic mothers (average age 29 ± 3 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 (10). 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 biological fathers did not have a history of atopic disease.

Nonatopic Controls. Twelve nonatopic, postpartum mothers (average age 30 ± 2 years, 39 ± 1 weeks of gestation) were enrolled in the study. Mothers and respective fathers of the newborns 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

Basophil Degranulation Assay. Three milliliters peripheral blood was diluted with PBS/EDTA (phosphate-buffered saline/ethylenediamine tetraacetic acid) to 6 mL. The 6 mL diluted blood was layered over Ficoll-Hypaque (6 mL, 1.085 g/mL) and then was centrifuged at $400 \times g$ for 30 min at 25°C . The cells were harvested and washed twice with pH 7.4 HEPES-ACM buffer and subsequently were incubated with different PGN concentrations (50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, 150 $\mu\text{g/mL}$), Df (10 $\mu\text{g/mL}$), or ACM buffer in a 37°C water bath for 30 min. The basophil degranulation index (DI) was determined by staining cells with alcian blue to visualize granule release. DI can be computed as follows:

$$\text{DI} = \frac{\text{Number of basophils in blank control group} - \text{Number of basophils in stimulation group}}{\text{Number of basophils in blank control group}} \times 100\%$$

Basophil Purification. Heparinized cord blood was mixed 5:1 with 6% dextran and incubated for 30 min at 37°C to destroy remaining erythrocytes. The cells were washed

twice with 50 mL of PBS (pH 7.2), 10 mM EDTA and then diluted in PBS/EDTA to 4 mL. The 4 mL cells were layered over the Ficoll-Hypaque (4 mL, 1.065 g/mL) and then centrifuged at $400 \times g$ for 30 min at 25°C . Basophils were concentrated at the 1.065 g/mL Ficoll interface, harvested, and washed twice with 50 mL of PBS/EDTA (11).

Granulocytes (from the cord blood of mothers) were preincubated with PBS/EDTA/0.5% BSA (bovine serum albumin) for 20 min at 4°C . Thereafter, cells were suspended in PBS/EDTA/0.5% BSA at a concentration of 10^7 per 80 μL and incubated with 20 μL of FcR Blocking Reagent, 10 μL PE-labeled CD203c, and 10 μL PerCP-labeled anti-CD45 per 10^7 cells for 10 min at 4°C with gentle shaking. The cells were then washed with PBS/EDTA/0.5% BSA. Basophils were sorted based on CD203c⁺CD45^{int+} staining.

Percent purity and recovery was determined by alcian blue staining of cells both before and after sorting (12). Viability was assessed using trypan blue exclusion.

Culture Conditions. Purified umbilical cord blood-derived basophils were washed with HEPES-ACM buffer and equilibrated at 37°C , 5% CO_2 for 15 min. The cells were then incubated with PGN, Df, or ACM buffer for an additional 8 h as above. Each group contained approximately 100,000 cells.

Flow Cytometry

To detect TLR2 expression, basophils obtained from cord blood were stained with FITC-labeled anti-TLR2 antibody before analysis by flow cytometry on a Fluorescence Activated Cell Sorting (FACS) Calibur machine (BD PharMingen, San Diego, CA, USA).

Detection of TLR2 mRNA by In Situ Hybridization

The TLR2 probe sequences were 5'-GCTAT GATGC ATTTG TTTCT TACAG TGAGC-3', 5'-TGGAT CATTG ACAAT ATCAT TGA CT CCATT-3', and 5'-CCCAG CGCTT CTGCA AGCTG CGGAA GATAA-3'. The presence of TLR2 mRNA was detected using conventional *in situ* hybridization methods (13). Image acquisition of exposed blots was performed using an Analysis System KS 400 3.0.

IL-4 Assays

Cell-free supernatants were harvested from 8 h cultures. IL-4 levels were quantified by ELISA as described by the manufacturer (14).

Statistics

Student *t*-tests, ANOVA, and Pearson correlation analyses were carried out using SPSS 13.0 software (NY) to assess

statistically significant differences between the treatment groups.

RESULTS

Basophil Stimulation

The DI was assessed following incubation of basophils with various PGN concentrations. Both 100 and 150 $\mu\text{g/mL}$ PGN enhanced a significant release of cytokines from peripheral blood-derived basophils of asthmatic mothers compared with controls (Table 1). Incubation with 150 $\mu\text{g/mL}$ PGN also increased the basophil DI in healthy controls, suggesting that 100 $\mu\text{g/mL}$ PGN was the optimal stimulation concentration for assessing differences between patient groups.

Df (10 $\mu\text{g/mL}$) also enhanced cytokines release from basophils isolated from asthmatic mothers compared with healthy controls ($p < .01$) (Table 1).

Assessment of Basophil Purity Following Isolation

Basophil concentrations between cord blood and the peripheral circulation are different because cord blood contains a proportion of immature basophils with underdeveloped granules (15). Therefore, characterizing the purity of the harvested basophils is particularly important. Reimer et al. (16) demonstrated that CD203c expression was detectable early during basophil development. This marker was a good alternative to identify basophil precursors. In addition, CD45 is expressed on basophils but not on mast cells. These basophil-restricted markers allowed for the positive selection and purification of umbilical cord blood-derived basophils using low side scatter of CD203c-PE⁺ and intermediate CD45-PerCP⁺-stained cells (Figure 1). Basophil integrity was further assessed by staining purified cells with alcian blue (Figure 2). Purity of basophils by Flow cytometry (FCM) with CD203c⁺CD45^{int+} markers was $93.00 \pm 5.02\%$ in asthma group and $92.01 \pm 4.94\%$ in healthy group.

TLR2 Expression

Baseline TLR2 expression was not significantly different in cord blood basophils from neonates born to either asthmatic or nonatopic mothers (Table 2). Following PGN stimulation (but not stimulation with Df), TLR2 expression was significantly upregulated in cord blood basophils from neonates born to asthmatic mothers compared with

expression levels observed in neonates born to nonatopic mothers ($p < .01$) (Table 2 and Figure 3).

TLR2 mRNA levels were characterized using *in situ* hybridization. Parallel to the TLR2 expression described above, TLR2 mRNA expression was increased following PGN stimulation (but not following Df stimulation) of cord blood basophils obtained from neonates born to asthmatic mothers ($p < .01$) (Table 3 and Figure 4A and 4B).

IL-4 Production Following PGN or Df Stimulation

The IL-4 levels produced by cord blood basophils from asthmatic mothers were significantly ($p < .01$) elevated compared with the levels detected for basophils isolated from nonatopic mothers following PGN or Df stimulation (Table 4).

A significantly positive correlation was observed between the DI of peripheral blood basophils from asthmatic mothers and that of cord blood-derived IL-4 following stimulation with either Df ($R = 0.70$, $p < .05$, Figure 5A) or PGN ($R = 0.68$, $p < .05$, Figure 5B).

DISCUSSION

The identification of TLRs has sparked a renewed interest in innate immunity because it is clear that these innate receptors play key roles in shaping the nature of the adaptive immune response, including atopic diseases (17). The primary ligand for TLR2 is PGN, a principal component of gram-positive bacterial cell walls. In addition to its capacity to recognize PGN, TLR2 can also bind lipoprotein, lipopolypeptide, teichoic acid, lipopolysaccharide, and zymosan (18). Furthermore, TLR2 also forms heterodimers with different TLRs allowing for the recognition of additional ligands (19).

Using a mouse model, it was demonstrated that the TLR2 analog receptor Pam3Cys induced the upregulation of Th2 cytokines and the downregulation of Th1 cytokines, thereby directing the elicitation of Th2-mediated responses (20). In patients with seasonal allergic rhinitis (Th2-mediated response), the levels of TLR3, TLR2, and TLR4 were observed to be upregulated, further supporting a role of TLR2 in atopic diseases (21). These data suggested that ligation of specific TLRs could exacerbate atopic diseases by activating Th2 immune pathways. Furthermore, Eder et al. (22) demonstrated that genetic variation in TLR2 was a major determinant of

TABLE 1.—Basophil DI following stimulation with PGN or Df.

PGN concentration ($\mu\text{g/mL}$)	50	100	150	Df (10 $\mu\text{g/mL}$)
Asthmatic group (DI%)	19.50 ± 5.19	$37.40 \pm 9.79^{***}$	$44.70 \pm 15.00^{***}$	$40.20 \pm 13.07^{***}$
Healthy group (DI%)	14.92 ± 5.55	19.92 ± 7.22	$29.92 \pm 10.26^*$	17.58 ± 7.06

Notes: Data are expressed as the mean percent $\pm$ SD. Experiments designed to define the dose-dependent effects of PGN on basophil degranulation demonstrated that 50 $\mu\text{g/mL}$ PGN had no significant effects compared with the 100 and 150 $\mu\text{g/mL}$ PGN doses that significantly increased basophil degranulation in allergic asthma patients compared with healthy controls. PGN doses of 150 $\mu\text{g/mL}$ also increased basophil DI in healthy controls, suggesting that the 100 $\mu\text{g/mL}$ PGN dose was adequate for assessing basophil degranulation in asthmatic patients. Cultures with Df also increased the basophil DI in asthmatic group, suggesting that Df enhanced cytokine releases from basophils isolated from asthmatic mothers compared with healthy controls ($p < .01$).

* $p < .01$ versus other conditions in each group using ANOVA analyses.

** $p < .01$ versus the healthy group using Student *t*-test.

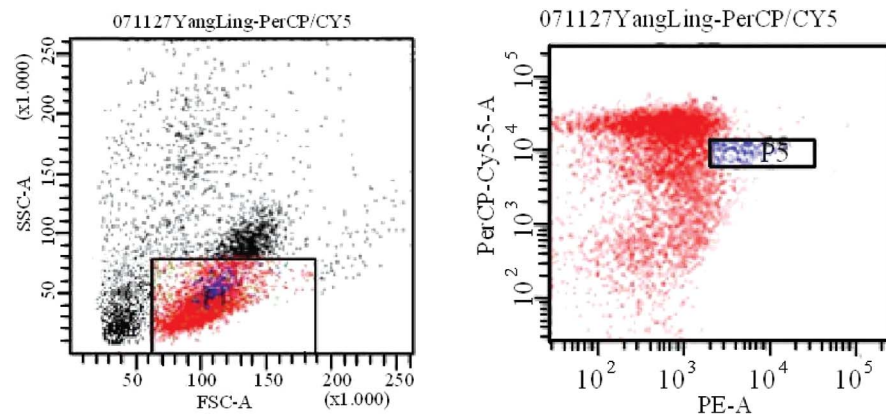


FIGURE 1.—Flow cytometric analysis of purified basophils. Purified basophils were examined by flow cytometry using (A) forward light scatter, low side scatter of (B) CD203c-PE and CD45-PerCP intermediate-stained cells (indicated by the blue stain in the P5 box).

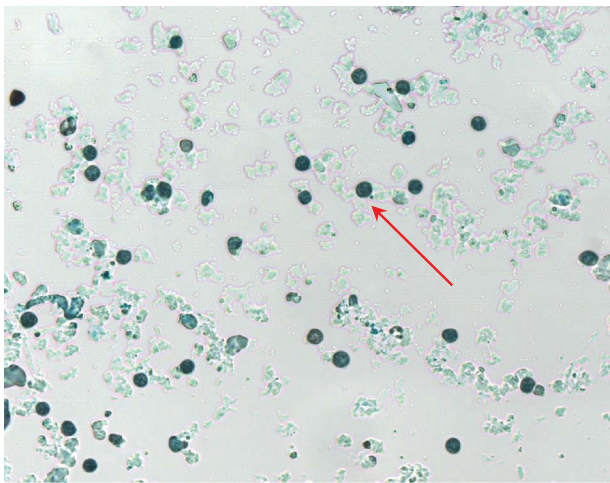


FIGURE 2.—Alcian blue staining. Basophil purity and integrity was assessed by staining harvested cells with alcian blue. This analysis revealed the presence of significant numbers of alcian blue positively stained (live cells, red arrow). Magnification $\times 400$.

TABLE 2.—Expression of TLR2 in cord blood basophils following stimulation with PGN or Df.

	Control group	PGN group	Df group
Asthma group	18.34 $\pm$ 3.63	46.24 $\pm$ 12.91*	21.50 $\pm$ 7.05
Healthy group	16.75 $\pm$ 6.40	21.78 $\pm$ 7.68	19.02 $\pm$ 4.31

Notes: The data are expressed as the mean percent $\pm$ SD.

* $p < .01$ compared with either the healthy group or the unstimulated basophils from the atopic group.

the susceptibility to asthma and allergies in children of farmers.

Similarly, the observations that Th2 responses were intricately involved in the progression of atopic diseases were reported. Recent data have revealed a role for basophils not only in the effector response, but also in the initiation of the inflammatory response through the production of IL-4, a key cytokine associated with the polarization of Th2-type responses (7, 23), suggesting that basophils are key players in allergic inflammation and potential therapeutic targets (2).

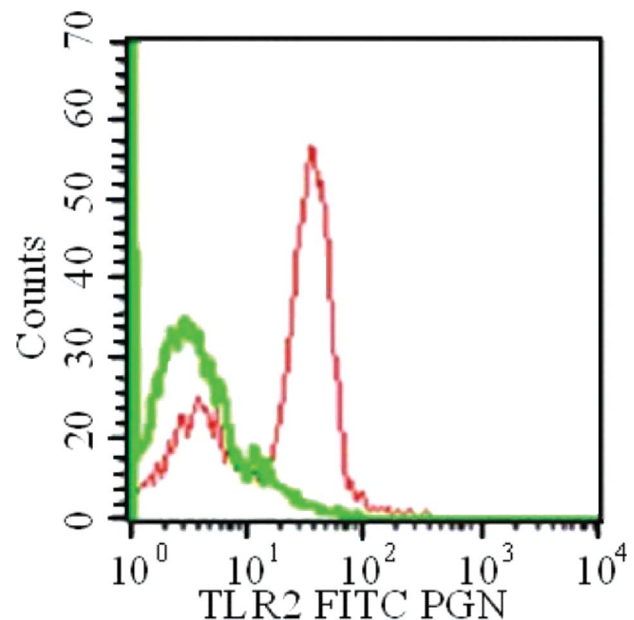


FIGURE 3.—Characterization of TLR2 expression. Basophils were isolated from atopic and nonatopic mothers and stimulated with PGN. After 8 h, cells were incubated with a FITC-labeled anti-TLR2 and staining was assessed by flow cytometry. The green line represents cells from one neonate born to asthmatic mother before PGN stimulation and the red line represents cells from the neonate after PGN stimulation.

TABLE 3.—TLR2 mRNA expression levels in cord blood basophils.

	Control group	PGN group	Df group
Asthma group	11.55 $\pm$ 4.32	39.53 $\pm$ 11.25*	14.01 $\pm$ 8.09
Healthy group	11.77 $\pm$ 4.03	16.98 $\pm$ 9.40	13.21 $\pm$ 6.28

Notes: The data are expressed as the mean percent $\pm$ SD.

* $p < .01$ compared with either the healthy group or the unstimulated basophils from the atopic group.

Basophil degranulation can result from the interactions with a myriad of stimulants, including cytokines and/or complement cleavage products. Yamamoto et al. (24) examined the effects of PGN using ovalbumin (OVA)-sensitized mice and demonstrated that mast cell degranulation resulted in increased eosinophil migration into the nasal mucosa and Th2-mediated exacerbation of allergic

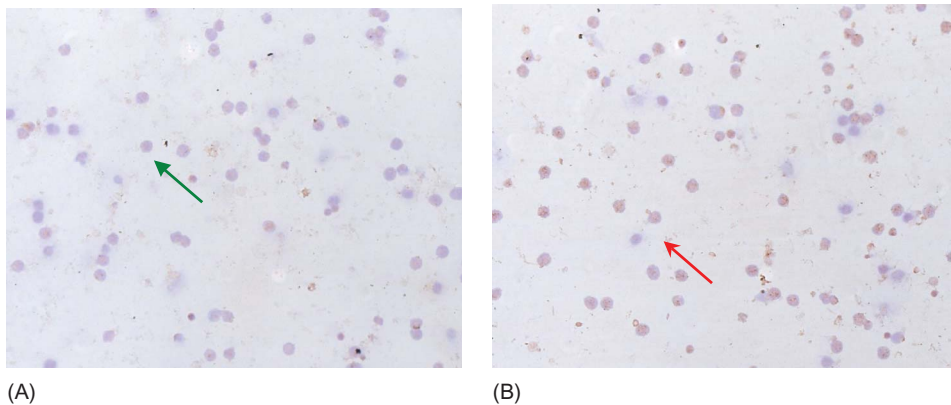


FIGURE 4.—*In situ* expression of TLR2 mRNA. Expression of TLR2 mRNA in cord blood basophils collected from one neonate born to asthmatic mother (A) before or (B) after PGN stimulation. TLR2-positive cells are stained brown (live cells, red arrow) and TLR2-negative cells are stained blue (live cells, green arrow).

TABLE 4.—IL-4 supernatant levels after stimulation with Df or PGN.

	Control group	PGN group	Df group
Asthma group	1.62 ± 0.69	3.27 ± 0.87*	3.01 ± 1.10*
Healthy group	1.41 ± 0.79	1.77 ± 1.02	1.51 ± 0.95

Notes: The data are expressed as the mean percent ± SD of observations per group (pg/mL).

**p* < .01 compared with either the healthy group or the unstimulated basophils from the atopic group.

rhinitis symptoms. It was also demonstrated in a mouse model that direct activation of mast cells through TLR2 or TLR4 (by respective microligands) contributed to innate and allergic immune responses and that TLR2—but not TLR4-dependent mast cell stimulation—resulted in mast cell degranulation and Ca^{2+} mobilization (25). However, few studies have described the roles of TLR ligand interactions on peripheral blood- or cord blood-derived basophils.

The role of respiratory infections on asthma development has attracted significant attention. A large number of asthma attacks, among adults and children, are precipitated by a respiratory infection that exacerbates asthma symptoms. Infection of the respiratory tract has emerged as an important clinical issue in stable patients with chronic asthma (26). Recently, Bisgaard et al. (27) found a significant association between bacterial infection of the

airways and acute wheezy episodes in young children. Therefore, bacterial infections may trigger many asthma attacks in children. Our experimental result may expound the mechanism. In this report, we studied basophil DI following PGN (a principal component of bacterial cell walls) stimulation and demonstrated that PGN-mediated signaling significantly elevated basophil degranulation in atopic patients compared with healthy controls. As PGN is a TLR2 ligand, we conclude that TLR2 ligation mediated the atopic response in our asthma patients.

After birth, the immune system usually shifts from Th2-polarized responses to Th1-mediated responses. If IL-4 levels were elevated during pregnancy (or right after birth), the elevated IL-4 would interfere with the development of Th1-mediated responses during infancy, thereby facilitating the elicitation of allergic responses (28). Sepp et al. (29) studied the association between changes in the intestinal flora and atopic disease in 1-year-old babies in Estonia (low incidence of atopic diseases) and Sweden (high incidence of atopic diseases), indicating that intestinal bacteria played a key role in the development of hereditary allergy. Lactobacilli and Eubacteria could be isolated from Estonian infants whereas *Clostridia* species were more common in Swedish infants (29). The PGN used in our study was extracted from *Bacillus subtilis*, suggesting

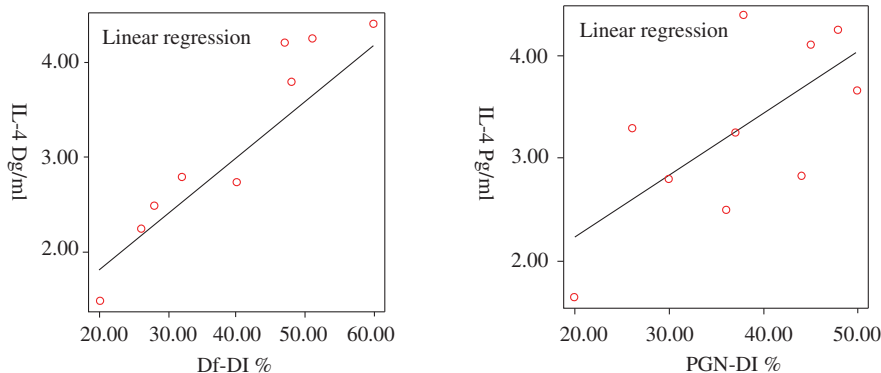


FIGURE 5.—Assessment of the IL-4 response. Cord blood basophils from 10 neonates born to asthmatic mothers were stimulated with either (A) Df or (B) PGN and the IL-4 supernatant levels were correlated to the DI. Each point on the graph represents the IL-4/DI response for an individual patient.

that intestinal microbial populations could interact (activate) basophils through TLR2 (especially in genetically predisposed infants) to release cytokines, for example, IL-4, associated with the progression of allergic diseases.

Data presented in this report demonstrated that cord blood basophils isolated from neonates born to atopic mothers had elevated TLR2 protein and mRNA expression levels. Following stimulation with PGN, TLR2 expression was significantly elevated in cord blood basophils from neonates born to asthmatic mothers compared with cord blood basophils isolated from neonates born to healthy mothers. Cord blood basophils from atopic mothers produced significantly higher levels of IL-4 following stimulation with either PGN or Df compared with IL-4 levels produced by cord blood basophils isolated from nonatopic controls. A significantly positive correlation was established between the basophil degranulation of peripheral blood basophils from asthmatic mothers and that of cord blood-derived IL-4 following stimulation with either Df or PGN, suggesting either the TLR2 or IgE receptors play a role in mediating atopic disease. Future studies will be carried out to determine whether anti-TLR2 treatments can prevent PGN-induced basophil activation as a means of developing treatments for atopic diseases.

DECLARATION OF INTEREST

The authors report no competing interests. The authors alone are responsible for the content and writing of this paper.

REFERENCES

- Falcone FH, Haas H, Gibbs BF. The human basophil: a new appreciation of its role in immune responses. *Blood* 2000; 96:4028–4038.
- Obata K, Mukai K, Tsujimura Y, Ishiwata K, Kawano Y, Minegishi Y, Watanabe N, Karasuyama H. Basophils are essential initiators of a novel type of chronic allergic inflammation. *Blood* 2007; 110(3):913–920.
- Prussin C, Metcalfe DD. 4. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2003; 111:S486–S494.
- Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. *Curr Opin Immunol* 2000; 12:624–631.
- Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J Exp Med* 1993; 177:605–611.
- Schroeder JT, MacGlashan DW, Kagey-Sobotka A, White JM, Lichtenstein LM. IgE-dependent IL-4 secretion by human basophils: the relationship between cytokine production and histamine release in mixed leukocyte culture. *J Immunol* 1994; 153:1808–1817.
- Oh K, Shen T, Le Gros G, Min B. Induction of Th2 type immunity in a mouse system reveals a novel immunoregulatory role of basophils. *Blood* 2006; 106:2011–2017.
- Sabroe I, Jones E, Usher L, Whyte M, Dower S. Toll-like receptor (TLR)2 and TLR4 in human peripheral blood granulocytes: a critical role for monocytes in leukocyte lipopolysaccharide responses. *J Immunol* 2002; 168:4701–4710.
- Bieneman AP, Chichester KL, Chen YH, Schroeder JT. Toll-like receptor 2 ligands activate human basophils for both IgE-dependent and IgE-independent secretion. *J Allergy Clin Immunol* 2005; 115:295–301.
- GINA Report, Global strategy for asthma management and prevention. 2006 Available at: <http://www.ginasthma.org>. the accessed date: 7/20/07.
- Yang L, Xu WG, Xu YP, Yao SH, Guo YS, Wang KM, Xiong Y, Zhu J. Method for umbilical cord blood-derived basophils by FCM. *Hybridoma* 2010; 29(4):367–370.
- Gilbert HS, Ornstein L. Basophil counting with a new staining method using alcian blue. *Blood* 1975; 46:279–286.
- Seibl R, Birchler T, Susanne L, Hossle JP, Gay RE, Saurenmann T, Michel BA, Seger RA, Gay S, Lauener RP. Expression and regulation of Toll-like receptor 2 in rheumatoid arthritis synovium. *Am J Pathol* 2003; 162:1221–1227.
- BMS225HS human IL-4 High Sensitivity ELISA manufacturer. Available at: http://www.bendermedsystems.com/bm_products/MAN/225HS.pdf.
- Kepley CL, Pfeiffer JR, Schwartz LB, Wilson BS, Oliver JM. The identification and characterization of umbilical cord blood-derived human basophils. *J Leukoc Biol* 1998; 64:474–483.
- Reimer JM, Magnusson S, Juremalm M, Nilsson G, Hellman L, Wernersson S. Isolation of transcriptionally active umbilical cord blood-derived basophils expressing Fc epsilon RI, HLA-DR and CD203c. *Allergy* 2006; 61:1063–1070.
- Janeway C, Jr, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002; 20:197–216.
- Zähringer U, Lindner B, Inamura S, Heine H, Alexander C. TLR2-promiscuous or specific? A critical re-evaluation of a receptor expressing apparent broad specificity. *Immunobiology* 2008; 213(3–4):205–224.
- Ishii KJ, Coban C, Akira S. Manifold mechanisms of Toll-like receptor-ligand recognition. *J Clin Immunol* 2005; 25(6):511–521.
- Redecke V, Hacker H, Datta SK, Fermin A, Pitha PM, Broide DH, Raz E. Cutting edge: activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J Immunol* 2004; 172(5):2739–2743.
- Fransson M, Adner M, Erjefält J, Jansson L, Uddman R, Cardell LO. Upregulation of toll-like receptors 2, 3 and 4 in allergic rhinitis. *Respir Res* 2005; 6(1):100–109.
- Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrlander C, Nowak D, Martinez FD. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004; 113(3):482–488.
- Mukai K, Matsuoka K, Taya C, Suzuki H, Yokozeki H, Nishioka K, Hirokawa K, Etori M, Yamashita M, Kubota T, Minegishi Y, Yonekawa H, Karasuyama H. Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity* 2005; 23(8):191–202.
- Yamamoto K, Kawamura I, Ito J, Mitsuyama M. Modification of allergic inflammation in murine model of rhinitis by different bacterial ligands: involvement of mast cells and dendritic cells. *Clin Exp Allergy* 2006; 36(8):760–769.
- Supajatura V, Ushio H, Nakao A, Akira S, Okumura K, Ra C, Ogawa H. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest* 2002; 109(10):1351–1359.
- Sutherland ER, Martin RJ. Asthma and atypical bacterial infection. *Chest* 2007; 132:1962–1966.
- Bisgaard H, Hermansen MT, Bønnelykke K, Stokholm J, Baty F, Skytt NL, Aniscenko J, Kebabdzic T, Johnston SL. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. *BMJ* 2010; 341:c4978.
- Moverare R, Elfman L, Stalenheim G, Björnsson E. Study of the Th1/Th2 balance, including IL-10 production, in cultures of peripheral blood mononuclear cells from birch-pollen-allergic patients. *Allergy* 2000; 55(2):171–175.
- Sepp E, Julge K, Vasar M, Naaber P, Björkstén B, Mikel-saar M. Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr* 1997; 86:956–961.

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