Toll-like receptor-mediated eosinophil–basophil differentiation: autocrine signalling by granulocyte–macrophage colony-stimulating factor in cord blood haematopoietic progenitors

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Summary
Eosinophils are multi-functional leucocytes that play a role in inflammatory processes including allergy and infection. Although bone marrow (BM) inflammatory cells are the main source of eosinophil-basophil (Eo/B) differentiation-inducing cytokines, a recent role has been demonstrated for cytokine induction through Toll-like receptor (TLR)-mediated signalling in BM progenitors. Having previously demonstrated that cord blood (CB) progenitors induce Eo/B colony-forming units (CFU) after lipopolysaccharide (LPS) stimulation, we sought to investigate the intracellular mechanisms by which LPS induces Eo/B differentiation. Freshly isolated CD34-enriched human CB cells were stimulated with LPS (and/or pharmacological inhibitors) and assessed for alterations in haematopoietic cytokine receptor expression and signalling pathways by flow cytometry, Eo/B CFU in methylcellulose cultures, and cytokine secretion using Luminex assays.

The LPS stimulation resulted in a significant increase in granulocyte–macrophage colony-stimulating factor (GM-CSF)-responsive, as opposed to interleukin-5-responsive, Eo/B CFU, which also correlated with significant increases in CD34+ cell GM-CSFRα expression. Functionally, CB CD34+ cells secrete abundant amounts of GM-CSF following LPS stimulation, via a p38 mitogen-activated protein kinase (MAPK)-dependent mechanism; this secretion was responsible for Eo/B CFU formation ex vivo, as shown by antibody blockade. We show for the first time that CB progenitor cells results in autocrine activation of p38 MAPK-dependent GM-CSF secretion facilitating Eo/B differentiation ex vivo. This work provides evidence that early life exposure to products of bacterial agents can modulate Eo/B differentiation, representing a novel mechanism by which progenitor cells can respond to microbial stimuli and so affect immune and inflammatory responses.

Keywords: cord blood; eosinophil–basophil; granulocyte–macrophage colony-stimulating factor; lipopolysaccharide; p38 mitogen-activated protein kinase.
LPS influences cord blood eosinophil–basophil differentiation

Introduction

Eosinophils are multi-functional leucocytes involved in a number of infectious and inflammatory processes, including allergic diseases.1 Eosinophil–basophil (Eo/B) lineage commitment is a highly regulated process that involves the common βc-subunit binding cytokines, in particular granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-5 (IL-5),2 which when co-linked to specific, high-affinity z chains, stimulate CD34+ progenitor cells in the bone marrow (BM) via activation of several signal transduction pathways.3 Both the janus kinase/signal transducer and activator of transcription (STAT) and mitogen-activated protein kinase (MAPK) pathways drive eosinophil differentiation of cord blood (CB)-derived progenitor cells.4,5 Although the production of GM-CSF and IL-5 is generally derived from inflammatory cells within the BM, it has recently been shown that BM-derived CD34+ cells secrete these cytokines after stimulation with Toll-like receptor (TLR) agonists.6–8

Toll-like receptors recognize microbial pathogens to activate intracellular signalling pathways during innate immune responses. TLR4 signalling is initiated by the binding of lipopolysaccharide (LPS) to the TLR-4/MD-2 receptor complex on cellular membranes leading to activation of multiple signalling pathways including nuclear factor-κB and MAPK, and resulting in inflammatory cytokine gene transcription.9 There are recent reports that haematopoiesis can be induced via direct TLR activation, independent of haematopoietic cytokines.6,7,10 Specifically, extrinsic microbial stimuli are able to ‘push’ progenitor cells toward a myeloid-committed cell fate.11 In relation to this, we have previously shown that TLRs are expressed by human CB progenitor cells and that stimulation with LPS, a prototypical TLR4 ligand, can induce Eo/B colony-forming units (CFU).12 Although the relationship to atopic predisposition was assessed previously,12 the primary focus of this work was to investigate the biological effects of LPS stimulation on CB progenitors; specifically, we aimed to delineate intracellular mechanisms by which TLR4 signalling may regulate Eo/B differentiation. As LPS signalling can influence BM progenitor cell differentiation both in vitro13 and in vivo14 with clinical implications related to survival from sepsis15 and risk of allergic disease,12 we evaluated LPS-activated intracellular mechanisms involved in Eo/B CFU formation12 of CB CD34+ cells. Our results provide novel insights into the molecular mechanisms associated with LPS-induced haematopoietic progenitor cell Eo/B differentiation.

Materials and methods

Cord blood collection

Pregnant mothers admitted to the Labour and Delivery ward at McMaster University Medical Centre, Hamilton, ON, Canada provided informed consent before delivery for CB donation. The CB samples were collected from otherwise healthy pregnant women as we were interested in investigating the mechanisms in CB CD34+ cells. Upon delivery, each CB sample was collected in a 60-ml syringe containing 2 ml heparin (1000 units/ml; Sigma, Mississauga, ON) and stored at 4°C until processing. This study was approved by the Hamilton Health Sciences/McMaster Faculty of Health Sciences Research Ethics Board.

Cord blood processing and CD34+ cell enrichment

Cord blood samples were depleted of erythrocytes using gravity sedimentation as previously described.12 To enrich the sample for CD34+ cells, the pellet was resuspended at a concentration of 5 × 10⁷ cells/ml in RoboSep Buffer (PBS containing 2% fetal bovine serum and 1 mM EDTA; Stem Cell Technologies, Vancouver, BC). The cells were transferred to a 5-ml Falcon polystyrene round-bottom tube (Becton Dickenson 2058, Franklin Lakes, NJ) and EasySep Negative Selection Human Progenitor Cell Enrichment Cocktail with CD41 depletion (Stem Cell Technologies) at a concentration of 50 µl/ml cells was added. The solution was mixed and incubated for 15 min at room temperature. The magnetic nanoparticles (Stem Cell Technologies) were added at a concentration of 50 µl/ml cells and incubated for 15 min at room temperature. The cell suspension was then brought to a total volume of 2.5 ml by adding RoboSep Buffer and the tube was placed inside the RoboSep Magnet (Stem Cell Technologies) for 10 min at room temperature. This sample was further enriched by placing the liquid portion in a new 5-ml tube and re-incubating the sample in the magnet for 10 min. The purity of CD34+ cells was between 85 and 90%.

LPS stimulation of CB CD34+ cells

Lipopolysaccharide from Escherichia coli 0111:B4 was purchased from Sigma and used at the optimal concentration of 10 µg/ml as previously reported.12 For stimulation studies, CD34+ enriched cells were stimulated with LPS overnight (37°C in 5% CO₂) in tissue culture plates (Falcon Plastics, Oxnard, CA) supplemented with RPMI complete (RPMI-1640, HEPES, Penicillin/Streptomycin and fetal bovine serum). After overnight incubation, cells were centrifuged and resuspended in FACS buffer for flow cytometry staining. Immunofluorescent staining for GM-CSFRα and IL-5Rα expression were performed as previously described.12

Phospho-flow to detect intracellular activation of signalling pathway molecules

Analysis of intracellular proteins followed a protocol that was described previously.16 Briefly, following incubation
(37°C in 5% CO₂) of enriched CB CD34⁺ cells with LPS for 5, 15, 30, 45 or 60 min, cells were fixed using PhosFlow CytoFix Buffer (BD Biosciences, Mississauga, ON, Canada), and then centrifuged for 10 min at 523,656 g. After washing, cells were permeabilized (PhosFlow Perm Buffer III; BD Biosciences) for 30 min on ice, washed with FACS Buffer (PBS, 0.1% sodium azide) then stained with a phycoerythrin-conjugated phospho-specific monoclonal antibody against p38 MAPK (pT180/pY182), extracellular signal-regulated kinase (ERK) 1/2 (pT202/pY204) or STAT5 (pY694), FITC-conjugated CD45 and peridinin chlorophyll protein-conjugated CD34 or isotype control, all purchased from BD Biosciences. The amount of phosphorylated p38, ERK 1/2 or STAT5 was calculated as stimulation index equal to the median fluorescence intensity (MFI)stimulated cells/MFIunstimulated cells.¹⁶

**Acquisition and analysis**

Acquisition was performed using an LSR II flow cytometer (BD Bioscience); 5 × 10⁵ events were collected for analysis. To enumerate CD34⁺ cells, we used an established multiparameter gating strategy as previously described.¹²

**Methylcellulose cultures**

Methylcellulose colony assays were completed as previously described¹² using enriched CB CD34⁺ cells at a plating concentration of 2 × 10⁵ cells/35 mm × 10 mm culture dish (Falcon Plastics) in duplicate.

Duplicate cultures were also grown in the presence of supernatant (1/10 final dilution in culture) for 14 days (5% CO₂, 37°C). The role of GM-CSF and IL-5 in supernatant stimulated Eo/B CFU formation was confirmed by adding 5 µg/ml anti-GM-CSF or anti-IL-5 (Peprotech, Rocky Hill, NJ) monoclonal antibodies to the supernatant-stimulated methylcellulose cultures. Eo/B colonies were defined as tight, round refractile cell aggregates of 40 cells or more, staining pink with eosin using Wright–Giemsa (Diff-Quik; Seimens, Newark, DE) and visualized by inverted light microscopy (Olympus CK 40, Olympus Co. Ltd, Tokyo, Japan).¹⁷

**CD34⁺ cell cytokine assays**

Freshly isolated CD34⁺ progenitor cells were cultured in RPMI complete medium in the absence or presence of LPS overnight. After overnight incubation (37°C, 5% CO₂), the cell-free supernatant was harvested and stored at − 80°C for subsequent analysis. Multi-analyte profiling was performed and acquired using a Perkin Elmer CS 1000 Autoplex Analyzer (Luminex XMAP Technology; Austin, TX). A bioplex cytokine assay was used that simultaneously measured the concentrations of GM-CSF and IL-5 in culture supernatant using a human cytokine/chemokine MILLIPLEX MAP kit (Millipore, Mississauga, ON, Canada). The assay sensitivities of these cytokines were 2-3 and 0-1 pg/ml respectively. All analyses were performed according to the manufacturer’s instructions. To determine the mechanism of GM-CSF secretion, CD34⁺ cells were stimulated with 50 µM STAT5 inhibitor¹⁵ or 50 µM PD98059¹⁹ (ERK 1/2 inhibitor), or 20 µM SB203580³ (p38 MAPK inhibitor) (Calbiochem, Cambridge, MA) or DMSO vehicle control for 45 min before LPS was added for overnight stimulation to induce GM-CSF secretion. These concentrations were found to be non-toxic to cells and of optimal dosage as determined by preliminary experiments.

**Statistical analysis**

Data were analysed using IBM SPSS STATISTICS version 20.0 (Chicago, IL) and presented in figures as mean ± SEM. Data that were not normally distributed were log transformed and subsequently analysed with the t-test to compare differences between two groups, or the analysis of variance with a Dunnett post hoc analysis for many groups. We applied the Mann–Whitney U-test to assess the sensitivity or robustness of the results, and the results were consistent. We set the criterion for statistical significance a priori at α = 0.05. All P-values were reported to two decimal places.

**Results**

**LPS stimulation of CB progenitor cells enhances GM-CSF-induced Eo/B CFU**

We have previously shown that CB CD34⁺ progenitor cells express functional TLR4 and respond to LPS stimulation through Eo/B CFU formation.¹² To confirm and extend those findings, freshly isolated CD34⁺ cells were stimulated with LPS and haematopoietic cytokines for 14 days in methylcellulose cultures. Although LPS alone could not induce Eo/B CFU formation, the combination of GM-CSF (P = 0.02) and LPS resulted in a significant increase in the number of enumerable Eo/B colonies (Fig. 1a). Although the mean value was increased, IL-5-responsive Eo/B CFU formation in the presence of LPS did not reach significance (Fig 1b).

**LPS-stimulated CB progenitors secrete significant amounts of GM-CSF**

We next assessed whether CD34⁺ cells stimulated with LPS secrete the Eo/B differentiation-inducing cytokines, GM-CSF and IL-5, using a bioplex cytokine assay. Although none of these cytokines was found in the culture medium, CD34⁺ cells alone do secrete ambiently low levels
of cytokines. As shown in Fig. 2(a), LPS induces significant levels of GM-CSF ($P = 0.02$) from CB progenitors. The mean level of IL-5 was increased in LPS-stimulated supernatant but this did not reach significance (Fig 2b).

LPS-stimulated CD34+ cells activate p38 MAPK signalling

Phospho-flow cytometry is an especially valuable tool for investigating signalling pathways in rare cell populations, like CD34+ progenitor cells. As it has been previously used to detect MAPK and STAT5 signalling pathways, which may be involved in cytokine secretion from TLR-stimulated CB progenitor cells, we investigated whether these pathways were activated by LPS stimulation of CB CD34+ cells. As shown in Fig 3, detectable levels of phosphorylated p38 MAPK were seen 5 min after LPS stimulation ($P = 0.046$) followed by a steady decline thereafter. Additionally, there was a trend to increased ERK 1/2 between 5 and 30 min ($P = 0.06$) with LPS stimulation. No significant differences in STAT5 expression, as evaluated over time, were detected in LPS-stimulated CB progenitor cells.

LPS-induced p38 MAPK is involved in GM-CSF secretion by CD34+ cells

As we show that LPS induces a significant increase in GM-CSF secretion from CB CD34+ cells (Fig 2), and that LPS can induce the rapid activation of p38 MAPK (Fig 3), we next assessed whether these pathways were involved in GM-CSF secretion by CB CD34+ cells. To do this, CD34+ cells were pre-incubated with MAPK inhibitors SB203580 (p38 MAPK inhibitor) or PD98059 (ERK 1/2 inhibitor) or a STAT5 inhibitor and GM-CSF secretion was assessed by Luminex technology. Although ERK 1/2 and STAT5 inhibition were found to reduce the mean percentage of CD34+ cells secreting GM-CSF after LPS stimulation, it was only the addition of SB203580, a specific p38 MAPK inhibitor, that significantly suppressed GM-CSF secretion ($P = 0.002$) compared with LPS-stimulated CD34+ cells alone (Fig 4).

LPS-induced GM-CSF secretion by CD34+ cells facilitates Eo/B CFU formation

We were next interested in whether LPS-induced GM-CSF could support Eo/B CFU formation. Indeed, as shown in Fig 5(a), the supernatant of LPS stimulated
CD34+ cells induced Eo/B CFU formation, which could be blocked by the addition of GM-CSF cytokine-specific monoclonal antibodies (P = 0.02); the reduction in Eo/B CFU formation by anti-IL-5 monoclonal antibodies was not significant. Morphology of the cells in the colonies indicated characteristic bi-lobed nuclei and eosinophilic granulation (Fig. 5b).

LPS stimulation of CB CD34+ progenitor cells increases GM-CSFRα expression

As alterations in Eo/B CFU production could be the result of modulation of haematopoietic cytokine receptors, CD34+ cells were stimulated with LPS overnight and then analysed for receptor expression using flow cytometry. As shown in Fig. 6, LPS stimulation of CB progenitors increased the sMFI of GM-CSFRα (P = 0.04). Although the mean level density of IL-5Rα was also increased, this value did not reach significance.

Discussion

Toll-like receptors are sentinels of the innate immune system, and have recently been ascribed a new role in the regulation of myeloid lineage commitment. Since haematopoietic processes are central to allergic inflammation and systemic bacteraemia, and given that LPS modulates CB progenitor cell and BM progenitor cell differentiation both in vitro and in vivo, we further investigated the potential intracellular mechanisms regulating LPS-induced Eo/B CFU formation in human CB CD34+ cells.

We show that LPS enhancement of Eo/B CFU is specific to GM-CSF-responsive CD34+ progenitor cells, as opposed to IL-5-responsive progenitor cells, and is also associated with preferential up-regulated expression of GM-CSFRα (Fig 6). Additionally, we show that CB CD34+ cells stimulated with LPS activate p38 MAPK signalling pathways, which are involved in the autocrine secretion of GM-CSF; this cytokine plays an important role in facilitating Eo/B CFU formation in vivo, as evidenced by antibody blockade. We had previously observed that in vitro Eo/B maturation of CD34+...
progenitors is accompanied by an increase in GM-CSF mRNA and protein in maturing colony cells;23 our current finding of increased expression of GM-CSFRz after LPS stimulation, and its association with increased functional responsiveness of these cells to GM-CSF in colony assays, provides an additional explanation for this autocrine effect, as others have also noted.24 In support of this, blocking signal transduction via GM-CSFRz through GM-CSF inhibition reduced Eo/B CFU formation. Whether or not secreted GM-CSF auto-regulates GM-CSFRz expression is unknown to us; however, we cannot refute this possibility because GM-CSF has been shown to alter the expression of its cognate receptor in peripheral blood eosinophils.25 Collectively, these findings highlight a role for GM-CSF/GM-CSFRz in LPS enhancement of Eo/B differentiation, which is consistent with previous observations on the development of myeloid cells26 and eosinophil progenitors.27

Stimulation by TLR has been shown to involve the activation of MAPK signalling pathways in human monocytes,9,28 macrophages,29 eosinophils,30 and CB progenitor cells.21 In relation to progenitor cells, we have previously shown that IL-5-stimulated or GM-CSF-stimulated peripheral blood progenitor cells undergo rapid phosphorylation of p38 MAPK within 1–5 min using phospho-ELISA.17 Although not in a kinetic study, Kim et al.21 also showed that in CB progenitors stimulated with TLR-9 agonists there is up-regulation of both p38 MAPK and ERK 1/2. Our findings therefore complement and extend the latter study, showing that significant

Figure 5. Lipopolysaccharide (LPS)-induced cytokines from CD34+ cells induce eosinophil–basophil colony-forming units (Eo/B CFU). (a) The supernatants of previously stimulated cord blood (CB) CD34+ cells were incubated alone or with anti-interleukin-5 (IL-5) or anti-granulocyte–macrophage colony-stimulating factor (GM-CSF) antibodies in methylcellulose culture for 14 days (37°C and 5% CO2). Eo/B cultures are tight, granular clusters of 40 cells or more. Data are presented as mean ± SEM of six supernatant-stimulated cultures. Significant findings were P < 0.05 and indicated on the graph. (b) Picographs were developed from cytopsins of Eo/B colonies. Eo/B cells were stained pink with eosin using DiffQuik solution. Images of Eo/B colonies and Eo/B cells are shown at 40 × and 400 × magnifications respectively. N.D. not detected.
phosphorylation of p38 MAPK is also detected in CB CD34+ cells stimulated with other TLR (LPS) agonists (Fig. 7).

While others have reported that BM-derived CD34+ cells respond to TLR stimulation with the production of cytokines including GM-CSF, IL-5Rα, the potential mechanism(s) of this secretion were not investigated. Our demonstration that blocking p38 MAPK signalling in CB CD34+ cells suppresses LPS-induced GM-CSF secretion is therefore novel. Related to this, Kim et al.21 have demonstrated that TLR9 stimulation of CB CD34+ cells activates the p38 MAPK and ERK 1/2 pathways involved in IL-8 secretion. Our data show for the first time that LPS-induced GM-CSF production, which facilitates Eo/B CFU formation, directly involves TLR4/p38 MAPK signal transduction in CB CD34+ cells. In this way, LPS is only one component of this autocrine effect, a co-factor in Eo/B CFU formation, which uses the production of GM-CSF through MAPK signalling pathways to induce Eo/B differentiation from CB CD34+ cells. This is in line with studies that have shown that p38 MAPK is an integral part of the TLR4 axis of signal transduction.31

We have previously shown that CB progenitor cells from high-atopic risk infants have reduced capacity for Eo/B CFU formation after LPS stimulation.12 It has recently been shown that children of atopic mothers have reduced TLR-dependent p38 MAPK signalling in their blood monocytes up to the age of 2 years.32,33 In light of our current results, we hypothesize that reduced CB Eo/B differentiation after LPS stimulation in high-atopic risk infants12 may be the result of reduced p38 MAPK-induced GM-CSF production by CD34+ cells, possibly related to epigenetic effects on p38 MAPK expression in utero. Along these lines, prenatal exposure to bacterial microflora (Acinetobacter lowffii F78) has been shown to prevent the development of allergy in offspring34 through microbial-induced epigenetic regulation of the IFN-γ promoter.35 Although the assessment of atopy was not the objective of this study because we were interested solely in the biological implications of LPS stimulation on human CB CD34+ cells, we are now in position to examine this hypothesis in prospective birth cohorts.

Multi-potent progenitor cells have been previously shown to express TLRs and respond to receptor ligation by production of myeloid committed cells;6,7,13 indeed, alterations in haematopoiesis may represent another mechanism for targeted redirection of the immune response to confront invading pathogens. For example, it

Figure 6. Stimulation of cord blood (CB) CD34+ cells with lipopolysaccharide (LPS) increases granulocyte-macrophage colony-stimulating factor receptor-α (GM-CSFRα) expression. CB CD34+ cells were stimulated with 10 μg/ml LPS overnight before being stained with antibodies to GM-CSFRα (a) or interleukin-5 receptor-α (IL-5Rα) (b). Histograms shown are representative of one experiment. Light-tinted histograms are isotype controls for the unstimulated and stimulated samples, and filled histograms are the receptor of interest in the unstimulated (black) and stimulated samples (dark grey). Data were also presented as the mean ± SEM of six experiments measured by specific median fluorescent intensity (sMFI12), which represents the number of receptors per cell (c). Significant findings were P < 0.05 and indicated on the graph.

Figure 7. p38 mitogen-activated protein kinase (MAPK)-dependent granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion by lipopolysaccharide (LPS)-stimulated CD34+ cells induces eosinophil-basophil colony-forming unit (Eo/B CFU) formation. LPS signalling in cord blood (CB) CD34+ cells induces rapid p38 MAPK phosphorylation (1). This activation results in the intracellular production of GM-CSF, which is secreted (2), and serves to induce Eo/B CFU ex vivo (3).
has been shown that sepsis is sometimes associated with neutropenia, accompanied by peripheral blood and BM myeloid progenitor cell mobilization and differentiation. In the case of eosinophils, there is a documented case of cryptococcal infection combined with sepsis, resulting in eosinophilia in a healthy individual. Likewise, LPS has been shown to influence haematopoietic dynamics through direct effects on progenitor cells, including rapid myeloid differentiation. Increased Eo/B CFU production after LPS stimulation of CB CD34+ cells may represent a mechanism through which haematopoietic progenitor cells or their resulting mature progeny can help to respond to invading bacterial species during acute infections. These mechanisms may also be operative in allergic (eosinophilic/basophilic) inflammation.

Our data are interesting in the context of the type of immune response that can be generated in response to bacterial agents. Of note, IL-5 is an eosinophil-specific inducing cytokine, whereas GM-CSF-responsive progenitors represent earlier stages of lineage commitment and therefore contribute to the development of several myeloid cells including Eo/B cells, macrophages and neutrophils. Therefore, the apparent skewing of the Eo/B progenitor population towards GM-CSF-responsive (Fig 1a), as opposed to IL-5-responsive, lineages (Fig 1b), with noted increases in GM CFU (data not shown), suggests that the progenitor response to LPS involves production of multi-cellular (Eo/B and GM) inflammatory responses to pathogens or allergens.

Although relatively high doses of LPS were used in the ex vivo culture system, this must be tempered by knowledge of the bio-availability of LPS in vivo. Physiologically, the fetus is exposed in vivo to LPS, because Gram-negative bacteria and associated LPS can be isolated from amniotic fluid in median concentrations of 0.05 μg/ml. Though the minimal concentration of biologically active LPS present within the intrauterine environment is unknown, soluble factors (e.g. sCD14) can modulate immune cell responses to LPS at 1000-fold lower concentrations than those observed in amniotic fluid. The LPS concentration that we used in the current studies is in line with other in vitro progenitor cell studies, which have found minimal progenitor cell responses to LPS below 10 μg/ml. In addition, Roy et al have demonstrated that endotoxin levels range between 1 and 6 μg/g house dust in rural and urban homes. Hence, the dose of LPS used here appears to be in the physiological range of natural LPS exposure.

We cannot conclude without addressing a couple of limitations of this study. First, the sample size used in this study appears to be small but we have completed the necessary sample size calculations, which show that as few as four CB samples are necessary to see differences in haematopoietic cytokine-induced CFUs. With this in mind, we are reassured of the significance of the findings and our interpretation that GM-CSF-mediated Eo/B CFU formation is an important pathway induced by LPS-stimulated CD34+ cells. Finally, there was a slight limitation with the type of LPS used for the study. We understand that this was not an ultrapure version of LPS, and therefore could be activating TLRs other than TLR-4. However, this study was not designed to investigate the TLR through which LPS signals, but instead was designed to determine the biological effect (e.g. activation of signalling pathways involved in Eo/B CFU formation) of LPS stimulation of CD34+ cells.

In conclusion, the novel autocrine mechanism of LPS-mediated Eo/B differentiation capacity shown herein points to the potential importance of TLR-mediated haematopoiesis in utero in relation to the development of allergic inflammation or immune responses to microbial stimulation. With interest increasing in p38 MAPK as a therapeutic target in inflammatory disorders, an understanding of the biology of TLR-mediated Eo/B differentiation may aid in the development of therapeutic interventions for infants at high atopic risk or for neonatal responses to infection.

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Disclosures

The authors declare no competing financial interests.

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