Cord Blood CD34+ Hemopoietic Progenitor Eosinophilic Lineage Commitment Assessed by Q-PCR

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Abstract

Rationale: We have recently demonstrated multiplex Q-PCR analyses of mRNA expression of key eosinophil-lineage commitment events in cord blood (CB) non-adherent mononuclear cells (NAMNCs) after stimulation with IL-5. We hypothesized that the majority of these events were occurring in the differentiating pluripotent CD34+ cell population.

Objective: To ascertain the kinetic patterns of expression of CB eosinophil-lineage specific genes, namely GATA-1, MBP, and IL-5Rα, in a purified CB CD34+ fraction.

Methods: CB mononuclear cells were isolated from random fresh and frozen samples, separated by magnetic cell-sorting into a highly- purified CD34+ population, and then incubated in the presence of 1 ng/mL rhIL-5. At various time-points post-stimulation, RNA was isolated, reverse transcribed, and expression of GATA-1, IL-5Rα, and MBP mRNA determined, utilizing multiplex Q-PCR. Relative expression ratios of stimulated and un-stimulated cells were calculated using the ΔΔCt method.

Results: Stimulation of CD34+ cells with IL-5 resulted in up-regulation of GATA-1 mRNA, peaking between 24 and 48h, an identical pattern to that seen with NAMNCs (r=0.963); MBP mRNA was up-regulated progressively, maximally at 96h (correlation with NAMNC, r=0.978). Total IL-5R mRNA was stable at all time-points, with differential expression of mRNA for IL-5R soluble and transmembrane isoforms.

Conclusion: Multiplex Q-PCR analyses of mRNA from CB CD34+ cells stimulated with IL-5 demonstrate sequential expression of critical eosinophil lineage-specific events, providing potential surrogate molecular markers of CB eosinophil differentiation.

Introduction

• The Denburg lab has previously identified that the number and phenotype of cord blood (CB) eosinophil/basophil (Eo/B) progenitor cell relate to atopic risk and infant clinical outcomes

• All previous evaluations utilized either 14 day methylcellulose colony assays or flow cytometry analysis

•We have previously demonstrated the kinetics of GATA-1 , MBP and IL-5R mRNA expression in CB non-adherent mononuclear cells (NAMNC) after IL-5 stimulation, and shown these to be in concert with subsequent Eo/B colony forming units (CFU's) in methylcellulose culture

•We had not examined the expression of these molecular markers in purified CD34+ cell fractions, and thus sought to confirm that the signals of eosinophil lineage commitment detected in the NAMNC fraction were indeed consistent with changes generated by these pluripotent progenitors.

Objective

• To ascertain the kinetic patterns of expression of CB eosinophil-lineage specific genes in response to IL-5 stimulation, namely GATA-1, MBP, and IL-5Rα, in a purified CB CD34+ fraction.
• To confirm that the NAMNC population can be utilized as a surrogate for the CD34+ cell line in the setting of IL-5 stimulation.

Methods

• CB mononuclear cells were isolated from random fresh and frozen samples, separated by magnetic cell-sorting into a highly-purified CD34+ population, and then incubated in the presence of 1 ng/mL rhIL-5.

• At 24, 48, 72h, and 1 week post-stimulation:
  • RNA extracted using RNeasy® Mini-Kit columns (Qiagen) according to manufacturer's instructions
  • DNA contamination removed using DNA Free kit® containing DNAse-1 buffer and DNAse-1 mix (Ambion) according to manufacturer's instructions
  • Total RNA in each sample was quantified using UV spectrophotometer
  • Reverse-transcription was completed with 2.97 mL random hexamer primers and 0.03 mL oligo (dT) primer (both 100ng/mL)

• Expression of IL-5Rα, GATA-1, and MBP were determined utilizing comparative Q-PCR in a multiplex reaction (Stratagene MX4000); Housekeeping gene was GAPDH

• IL-5Rα isoform analysis completed in Dr. Steven Ackerman's laboratory (University of Illinois at Chicago); housekeeping gene was beta-2-microglobulin

• Normalized relative expression ratios between stimulated and un-stimulated cells were calculated using the delta-delta Ct method ($^{2-\Delta\Delta Ct}$)

Results

• Stimulation of CBMNC with rhIL-5 resulted in the following patterns of expression:

• GATA-1: Up-regulation that peaked at 48hrs followed by down-regulation at 72hrs; excellent correlation with NAMNC fraction (r=0.963; p=0.034)

• IL-5Rα: Stable expression throughout all time-points when evaluating total expression; this was later sub-analyzed to show relative contributions of the transmembrane vs. the soluble isoforms
  • Transmembrane – initially comprises the majority of IL-5Rα expression, and is later down-regulated
  • Soluble –steady up-regulation, peaking at 72hrs*
  • Time course changes statistically significant (p=0.01; ANOVA on ranks)

• MBP: Up-regulation in a slowly progressive pattern, with maximal up-regulation at 72hrs; good correlation with NAMNC fraction (r=0.988; p=0.012).

* The authors gratefully acknowledge the assistance of Drs Steven Ackerman and Jian Diu of the University of Illinois at Chicago with this analysis.

Results - continued

Figure 1: Kinetic mRNA expression patterns of GATA-1, MBP and IL-5Rα (Total) from CB CD34+ purified cell populations in response to stimulation with rhIL-5

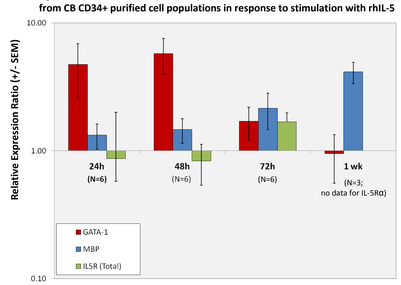


Figure 2: Kinetic mRNA expression of IL5-Rα (total) from CB CD34+ cell fraction stimulated with rhIL-5

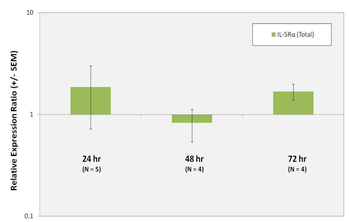
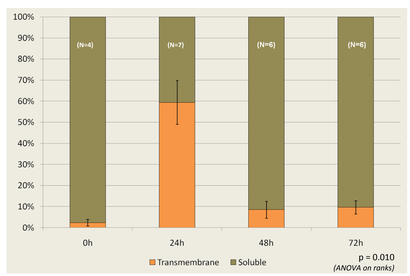


Figure 3 : Kinetic changes in the mRNA expression of the TM & Sol-IL-5Rα isoforms in CB CD34+ cells incubated with IL-5 (shown as percent of total IL-5Rα)



Discussion

In the current study, we saw an appropriate up-regulation of GATA-1 mRNA expression upon stimulation of a purified CD34+ cell population with IL-5, suggesting activation of cellular events leading to eosinophil development. Our results suggest that IL-5 is responsible for driving the maturation of the eosinophil lineage, with early peaking of GATA-1 followed by later up-regulation of MBP

We also found that the mRNA expression of IL-5Rα in CB CD34+ population remained apparently stable after stimulation with rhIL-5, but subsequent analysis which provided the contributory roles of the soluble and transmembrane variants of the IL-5Rα subunit revealed that this was in fact due to progressive down-regulation of the Tm-IL-5Rα isoform, and progressive up-regulation of the soluble isoform. This is in keeping with the work from others who have shown a similar switch from the predominantly soluble isoform to Tm-IL-5Rα in early IL-5-driven eosinophil development from human umbilical cord CD34+ cells32, but the predominant expression of the sol-IL-5Rα isoform in mature eosinophils.

Conclusions

- **Analysis of mRNA from cord blood CD34+ cells stimulated with IL-5 demonstrates sequential expression of critical Eo/B lineage-specific events**
- **These kinetic patterns mirror those seen with CB NAMNCs similarily stimulated with IL-5.**
- **Q-PCR analysis of the expression of these genes can be used as a surrogate, molecular marker of CB Eo/B differentiation in basic research and clinical studies**

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