Molecular Markers of Eosinophilopoiesis: Multiplex Q-PCR Analysis of GATA-1, MBP and IL-5 Receptor mRNA Expression in Peripheral Blood

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Abstract

Rationale: Using colony assays and flow cytometry, we have shown that eosinophil/basophil (Eo/B) progenitor phenotype and function are associated with both atopic risk at birth and early childhood clinical outcomes. We have also recently demonstrated that real-time polymerase chain reaction (Q-PCR) can reveal kinetic patterns of expression in cord blood (CB) of several Eo/B lineage-specific genes, specifically GATA-1, MBP and IL-5Rα, as surrogate molecular markers of Eo/B differentiation. These same methods have yet to be established in peripheral blood (PB) samples.

Objective: To utilize Q-PCR to determine the kinetic patterns of expression of CB Eo/B-lineage specific genes in PB, in order to evaluate surrogate markers of Eo/B differentiation.

Methods: PB non-adherent mononuclear cells (PB NAMNC) were isolated from random fresh samples, and incubated in the presence of IL-5. At 24, 48 and 72h post-stimulation, RNA was isolated, reverse transcribed, and expression of IL-5Rα, GATA-1, and MBP was determined utilizing comparative Q-PCR in a multiplex reaction. Relative expression ratios of stimulated to un-stimulated cells were calculated using the delta-delta Ct method.

Results: Stimulation of PB NANMC with IL-5 resulted in an up-regulation of GATA-1 expression, peaking at 24h, with a slower return to baseline expression than that observed in CB. MBP expression was minimally altered at all time points, compared to CB, where slow up-regulation, maximal at 72h, had been observed. There was completely stable expression IL-5Rα, similar to that seen in CB.

Conclusion: Multiplex Q-PCR analysis of mRNA from PB demonstrates expression of critical Eo/B lineage-specific events. Further investigation of the validity and utility of Q-PCR analyses of PB for surrogate, molecular markers Eo/B differentiation and their relationship to atopy and atopic outcomes are underway.

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Objectives

- To determine whether mRNA for GATA-1, MBP and IL-5Rα can be analyzed via a multiplex Q-PCR reaction in PB samples
- To establish the kinetics of PB Eo/B lineage surrogate marker mRNA expression in response to IL-5 stimulation

Methods

- PB NAMNCs were isolated from random fresh samples, and incubated in the presence of rhIL-5 (1 ng/mL) (7 to 14 million cells included for each condition)
- At 24, 48 and 72h 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
  - 25 µg RNA reverse-transcribed for each sample (volume calculated via total RNA quantification)
  - Reverse-transcription 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 was determined utilizing comparative Q-PCR in a multiplex reaction (Stratagene MX4000); housekeeping gene was GAPDH
- 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 PBMNC with rhIL-5 resulted in the following patterns of expression:
  - GATA-1: Up-regulation that peaks between 24 and 48hrs and diminishes at 72hrs, down-regulated by 1 week
  - IL-5Rα: Stable expression throughout all time-points
  - MBP: Stable expression that is slightly down-regulated compared to baseline, up-regulation by 1 week
- This differed slightly from the patterns seen in cord blood, as summarized in the introduction

Figure 1: Relative expression of GATA-1, IL5-R and MBP mRNA in PB NAMNC'S stimulated with IL-5

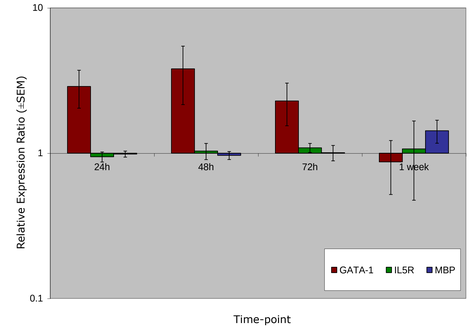


Figure 2A: Relative Expression of GATA-1 mRNA in IL-5 stimulated NAMNC's: Cord Blood vs. Peripheral Blood

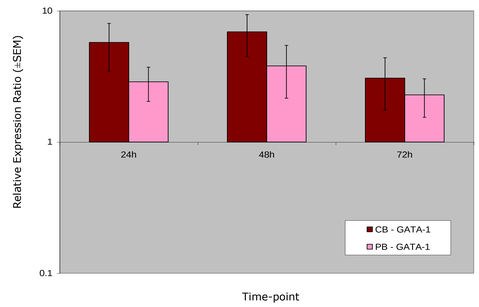
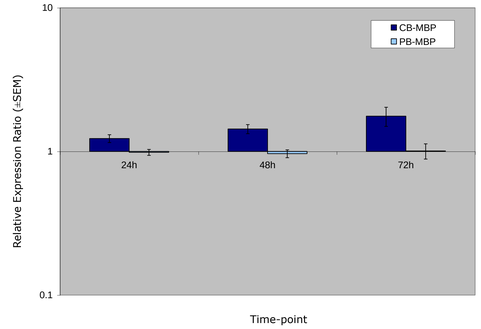


Figure 2B: Relative Expression of MBP mRNA in IL-5 stimulated NAMNC's: Cord Blood vs. Peripheral Blood



Introduction

- We have previously identified that the number and phenotype of cord blood (CB) eosinophil/basophil (Eo/B) progenitor cells relate to atopic risk and infant clinical outcomes
- Further, we have recently demonstrated that real-time polymerase chain reaction (Q-PCR) can reveal kinetic patterns of expression in CB of several Eo/B lineage-specific genes, specifically GATA-1, MBP and IL-5Rα, as surrogate molecular markers of Eo/B differentiation
- With IL-5 stimulation, CB non-adherent mononuclear cells (NAMNC's) demonstrated up-regulation of GATA-1 mRNA expression that peaked between 24 and 48hrs, followed by diminished expression at 72hours; stable expression of IL-5R mRNA and slow but steady up-regulation of MBP to a 2-fold degree by 72 hours
- These same methods have yet to be established in peripheral blood (PB) samples; indeed, it is uncertain whether similar findings would be observed given the lower percentage of progenitors in PB compared to CB, or if these markers of Eo/B lineage development can be detected in PB

Discussion

The patterns of expression of each of the genes evaluated were similar to that seen in cord blood, but with important differences.

Firstly, GATA-1 mRNA expression was not up-regulated to the same degree as in cord blood, and was slower to diminish to baseline levels. Secondly, MBP gene expression was not up-regulated in the initial time frame studied (72 hours).

Both of these observations could reflect the fact that the peripheral mononuclear cell population is less plastic and responsive to cytokine stimulation than the cord blood population. One would expect a lower percentage of Eo/B progenitors in PB, thus a smaller fraction of the total NAMNC population will respond to IL-5 stimulation with activation of eosinophil-lineage-specific transcription factors such as GATA-1, or new expression of genes for eosinophil products such as MBP. Since mature eosinophils would not be expected to be present in the NAMNC layer, the only cells capable of generating such a response would be the circulating Eo/B progenitor.

Preliminary evaluation of a 1 week incubation time-point indicates a down-regulation of GATA-1 expression, and detectable increases in MBP expression to approximately 1.5 fold baseline expression, similar to that seen in CB by 72 hours.

Conclusions

- **mRNA of GATA-1, IL-5Rα, and MBP can be evaluated from peripheral blood in a multiplex Q-PCR reaction.**
- **Further investigation of the relationship between the kinetics of these markers in NAMNC's and purified CD 34+ cell fractions is underway**

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