

A rapid new procedure for basophil isolation from human peripheral blood

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Introduction

Typically, basophils comprise < 1% of peripheral blood leucocytes in non-allergic, healthy humans. Phenotypically, basophils are distinguished from eosinophils by the expression of FcεR1, the hi-affinity receptor for IgE. Activation of basophils results in degranulation and release of histamine along with other immune modulators. Through these mechanisms, basophils play a pathogenic role in allergic inflammatory responses and parasitic infections.

Basophil research has been limited by the difficulty in isolating pure basophils in large numbers. Current protocols for their isolation are time-consuming, involving multiple steps and often require special equipment such as elutriators. Prolonged manipulation of basophils can lead to undesirable activation and spontaneous histamine release. Moreover no appropriate cell lines or animal models are available for studying basophil functional properties.

We describe a rapid and simple method for the enrichment of basophils from small quantities of normal fresh blood. The procedure does not require Ficoll and yields high basophil purity, recovery and viability. Blood was collected with heparin and red blood cells (RBC) were removed by HetaSep™ sedimentation. The use of HetaSep™ eliminated the requirement of careful layering over Ficoll™. The basophils were then enriched using immunomagnetic, column-free negative selection (EasySep®). Briefly, unwanted cells were specifically labeled with dextran-coated magnetic particles using a cocktail of bispecific tetrameric antibody complexes (TAC). The tube containing labeled cells was placed into a magnet. The supernatant containing the unlabeled basophils was collected leaving labeled cells behind in the magnet. The entire separation procedure has been automated with a pipetting robot (RoboSep®).

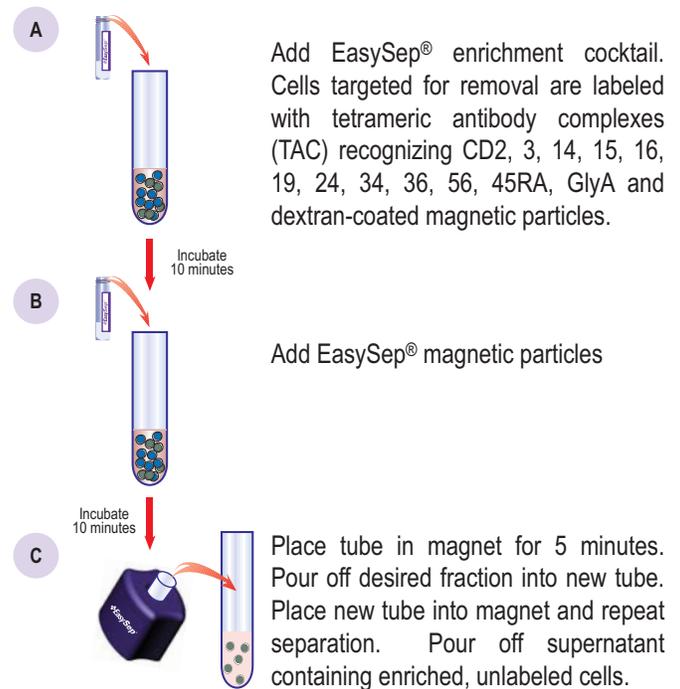
The method described will enable the simple acquisition of purified quiescent basophils, in sufficient numbers for further study.

Methods

HetaSep™ sedimentation for removal of RBC:

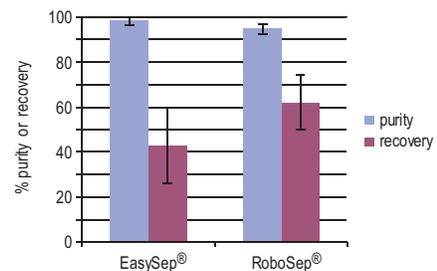
Whole blood was collected in a blood collection tube containing heparin. One part HetaSep™ was added to 5 parts blood and mixed well. The tube was centrifuged for 6 minutes at 110 x g with the brake off and then left to sit an additional 10 minutes at room temperature. The plasma layer containing the nucleated cells was removed without disturbing the RBC layer. This fraction was washed twice with cold buffer (PBS with 2% FBS plus 1mM EDTA). At least one of these washes incorporated a slow spin (120 x g, 10 minutes) to remove platelets. Cells were resuspended at 5 x 10⁷ cells/mL. The starting cell number range per experiment was 2.5 – 32.5 x 10⁷.

Figure 1. EasySep® procedure for column-free enrichment



Results

Figure 2. % Purity and % Recovery of Basophils enriched by negative selection using manual EasySep® or RoboSep®



Purities determined by flow cytometry. All samples gated on CD45⁺, viable (PI negative) cells and defined as CD123⁺ IgE⁺. Starting basophil frequency post-HetaSep™ was 0.9% ± 0.3. Values are expressed as means ± 1 sd. (n= 7 for EasySep®; n=5 for RoboSep®) Recoveries calculated from start cell suspension after HetaSep™.

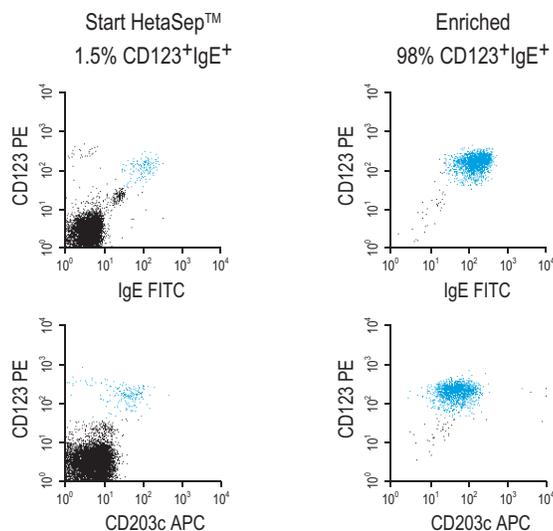
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Results (continued)

Figure 3. Typical FACS plots before and after enrichment of basophils (plots show viable (PI negative) cells gated on CD45⁺)

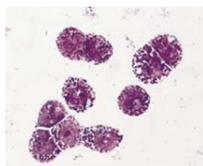


Functional studies

CD63 is not present on the membrane of resting basophils but appears on the cell surface during basophil degranulation. CD203c is found only on basophils in peripheral blood. It is expressed at low to intermediate levels on resting basophils and upregulated upon activation.

Gibbs *et al.*^{*}, using flow cytometry data, showed similar levels of CD63/CD203c expression between whole blood and unstimulated basophils that had been enriched by the described method. After 10 minutes stimulation with anti-IgE, increased levels of both CD63 and CD203c were demonstrated. Furthermore they showed that the enrichment procedure yielded basophils with normal responses to anti-IgE including histamine release and increased IL-4 and IL-13 mRNA expression.

Figure 4. Cytospin preparation stained with May-Grünwald showing fully granulated basophils in an enriched sample



Conclusions

- Basophils that are fully functional, quiescent and viable can be enriched from HetaSep™ treated whole blood using EasySep®. No columns are required. Entire procedure takes 90 minutes on average.
- Basophil enrichment can be fully automated using RoboSep®.
- No layering over Ficoll™ or lysis step is required to achieve high purity, viability (>98%) and recovery of basophils.
- Cell-surface CD203c/CD63 expression was not elevated in enriched cell samples as compared to whole blood*.
- Basophil function was not impaired by enrichment procedure as determined by IgE-mediated histamine release*.
- Enriched cells have typical basophilic morphology with intact granules.
- On average, approximately 16,000 basophils are recovered per mL of blood by the described method (range of 37 - 50% recovery from whole blood start).
- The rapidity and reproducibility of this method will facilitate the acquisition of basophils for numerous ex-vivo assays.

Reference:

*Gibbs BF, Papenfuss K. and Falcone FH. A rapid two-step procedure for the purification of human peripheral blood basophils to near homogeneity. *Clinical and Experimental Allergy* 2008 Mar; 38(3):480-5 E.pub 2007 Aug 14

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