A New Approach to an Old Dye for Distinguishing Basophils by Flow Cytometry or Teaching an Old Dye New Tricks

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Abstract:

Historically, the enumeration of the basophil population in a lysed blood sample has presented a problem. Typically, lymphocytes, monocytes, polymorphonuclear cells and eosinophils separate into reasonably distinct clusters of events when collected on forward scatter (FS) versus side scatter (SS) presentations on the flow cytometer. The basophil population, however, is commingled with the lymphocyte population, in the light scatter plot, making enumeration or isolation of basophils impossible. The method herein identifies a simple flow cytometric research method to separate the basophils from other cells in a lysed blood preparation and to obtain accurate basophil counts, without the vagaries of complicated gating strategies or algorithm constructs.

Anticoagulated whole blood was incubated with Astrazon Orange G (Basic Orange 21). The blood was then lysed and fixed on an automated preparation system and analyzed on a flow cytometer. Data was acquired for FS, SS and Astrazon Orange G fluorescence using a single 488 nm laser line. The resulting two Astrazon Orange G fluorescence populations, when gated into light scatter, represent distinct white blood cell clusters by light scatter. One fluorescence population comprises lymphocyte and monocyte populations, and the other fluorescent population includes polymorphonuclear populations (neutrophils, basophils, and eosinophils).

In summary: This method provides a simple, inexpensive research method to clearly resolve basophils from the remaining white blood cell populations in a lysed blood cell preparation, by flow cytometry.

Introduction:

Various methods are used to distinguish lymphocytes from basophils so that the two cell types in the cluster can be identified.

One method involves the use of subjecting the individual cells to an RF current source and measuring the electrical impedance change caused by the various cells in the total white blood cell population. In this method, the information from the DC volume (y-axis), light scatter (x-axis), and RF impedance (z-axis) are plotted in a three dimensional scatterplot. When the three dimensional scatterplot is examined in the third dimension, the basophils have a greater change in RF impedance, and are therefore adequately separated to facilitate an enumeration.

A second method involves the use of various combinations of fluorescent dyes and light scatter. Sakata and Kuroda (US Patent #5,296,378) discuss the use of Neutral Red and either Astrazon Orange G (also known as Basic Orange 21) or Auramine O in enumeration of white blood cells. Their method clearly states that two dyes must be combined to achieve a five-part classification of leukocytes.

A third method involves the use of surface staining monoclonal antibodies.

Yet another method involves selective lysing to differentiate basophils from other cell types.

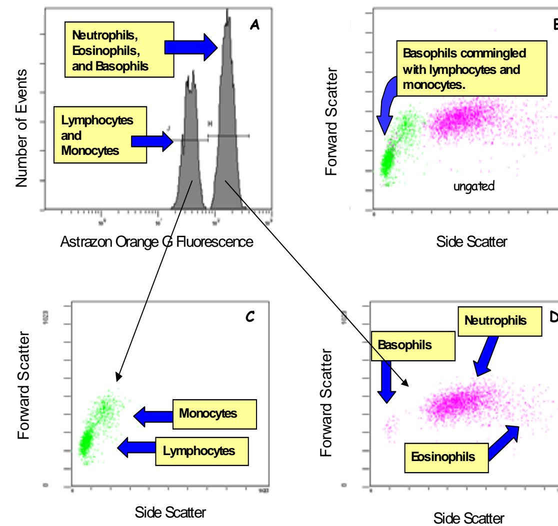
An easy method to more clearly resolve the basophils from other cells having similar characteristics is needed. This method of analysis of cells by flow cytometry can fully separate the basophils from lymphocytes, thereby making enumeration and isolation of basophils possible.

Materials and Methods:

- Samples should be less than 24 hours old for optimal results.
- 6 ul Astrazon Orange G (AOG) solution was mixed with 100 ul EDTA anticoagulated whole blood.
- The cell mixture was processed using a T-Q-Prep™ Sample Preparation Workstation and ImmunoPrep™ Reagent System (Beckman Coulter, Inc., Miami, FL).
- The prepared sample was analyzed on an FC500™ Series Flow Cytometer (Beckman Coulter, Inc., Miami, FL) with 488nm excitation.
- Fluorescence emission was measured through a 525/10 nm BP filter.
- Monoclonal antibodies were used to confirm cell populations.

Results: Gating Strategy

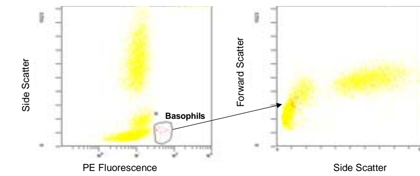
Figure 1



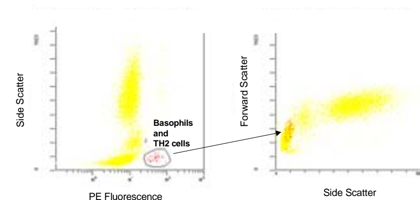
White blood cells within a whole blood sample treated with AOG differentially absorbed the dye. Cells stained with AOG were lysed and fixed and histograms were collected. Representative histograms of dye-stained leukocytes comprising a 5-part differential are shown. AOG fluorescence identified two peaks/populations of cells (Figure 1A). A light scatter plot was collected to identify four distinct cell populations with the fifth population (basophils) fully commingled with the lymphocyte population (Figure 1B). Light scatter plots gated from the less fluorescent AOG cell population and from the more fluorescent AOG population identify lymphocytes and monocytes (Figure 1C) and basophils, neutrophils and eosinophils, (Figure 1D) respectively.

Results: Monoclonal Antibodies

Figure 2



Monoclonal Antibody CD203c (clone 97A6) identifies resting and activated basophils and their position in a histogram.



Monoclonal Antibody CD294 (CRTH2: clone BM16) identifies basophils and TH2 cells and their position in a histogram.

Conclusions:

- Basophils can easily be separated from lymphocytes and monocytes using the described procedure.
- Basophils can be enumerated by flow cytometry using Astrazon Orange G.
- Neither complex gating nor algorithm constructs are required for this method of cell separation.
- Basophils can easily be sorted for other research purposes.
- This method is quick, easy, and inexpensive and provides a mechanism for counting or sorting basophils in whole blood.