Untouched isolation of functionally unaffected neutrophils from whole blood within 20 minutes

Claudia Zyntek, Jürgen Schmitz, and Gregor Winkels | Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

Human neutrophils are usually obtained by density gradient centrifugation. However, this method is exceedingly time-consuming and frequently results in neutrophil populations that are contaminated by other leukocytes, mainly eosinophils.

With the new MACSxpress Technology, untouched human neutrophils can be isolated from anticoagulated whole blood within 20 min. While erythrocytes are aggregated and sedimented and non-target cells are removed by immunomagnetic depletion with MACSxpress Beads, putting untouched cells at high purity and recovery. As one of the first lines of defense, neutrophils express a broad array of receptors and adhesion molecules making them highly susceptible to non-specific activation. To evaluate a possible influence of the newly developed separation process, neutrophils enriched by MACSxpress Technology were analyzed for the expression of different activation and adhesion markers and their capability to migrate upon different stimuli (Figs. 2 and 4). In addition, neutrophils were tested for their phagocytic capacity by analyzing the uptake of FITC-labeled E. coli (Fig. 3). To determine whether the separation process induces unwanted activation of neutrophils, we studied the spontaneous production of reactive oxygen species (ROS) and looked also for the level of ROS upon stimulation (Fig. 6). Flow cytometry analysis of neutrophils enriched by MACSxpress Technology shows that activation in response to stimuli does not lead to increased induction of apoptosis (Fig. 7).

Methods

The MACSxpress Neutrophil Isolation Kit enables the untouched isolation of functionally unaffected neutrophils from whole blood. Whole blood is incubated with MACSxpress Neutrophil Isolation Cocktail for 5 min at room temperature. Then the tube is placed in the magnetic field of a MACSxpress Separator (Fig. 1A) for 15 min. With the tube inside the strong magnetic field, the supernatant, containing the unlabelled target cells, is collected and transferred into a new tube Magnetically labelled target cells as well as aggregated erythrocytes are sedimented by centrifugation (red line) were characterized by flow cytometry for the viability (98±1%) of enriched neutrophils (n=6). The recovery by MACSxpress Technology compared with whole blood neutrophils, by other leukocytes, mainly eosinophils.

Results

1 Neutrophils are efficiently enriched from whole blood by MACSxpress Technology

Peripheral blood cells (Fig. 3, black line), neutrophils enriched by MACSxpress Technology (blue line), and cells isolated by density gradient centrifugation (green line) were characterized by flow cytometry for the expression of different adhesion-, migration- and activation-associated markers. Shown are histogram overlays following exclusion of dead cells and gating on neutrophils identified as CD15^+ cells. CD11b, CD24, and CD282 expression was slightly increased in neutrophils enriched by MACSxpress Technology compared with whole blood neutrophils, whereas cells enriched by density gradient centrifugation showed a higher increase. Expression of CD181, CD11b, CD24, and CD282 was reduced following density gradient centrifugation but not MACSxpress Enrichment, showing that adhesion/chemotactic and homing receptors are only slightly affected by the magnetic cell isolation process. Pattern recognition receptors CD282 and CD284 (not shown) were only slightly if at all influenced by the magnetic cell isolation process.

2 Expression of various marker proteins in neutrophils enriched by MACSxpress Technology

Enriched neutrophils were cultured for 3 h at 37 °C and then analyzed by flow cytometry for the expression of apoptotic markers (Fig. 7). Neutrophils undergoing early apoptosis were identified as Annexin V^+ and dead cells late apoptotic cells as Annexin V^+. No difference in expression of apoptotic and dead cells was found as well as Annexin V^+ and V^+ (Fig. 7).

3 Migration capacity of neutrophils toward fMLP is not influenced by cell isolation

Neutrophils were isolated from whole blood by MACSxpress Technology or density gradient centrifugation. Cells were incubated with FITC-labeled E. coli for 5 min at 37 °C and then analyzed by flow cytometry (Fig. 5). Flow cytometry analysis of neutrophils enriched by MACSxpress Technology shows that activation in response to stimuli does not lead to increased induction of apoptosis (Fig. 7).

4 Isolation of neutrophils does not alter phagocytic capacity

Neutrophils were isolated from whole blood by MACSxpress Technology or density gradient centrifugation. Cells were incubated with FITC-labeled E. coli and stained with anti-CD11b, CD24, and CD282. Phagocytic activity of neutrophils is shown as relative percentage of ingested bacteria. Phagocytic activity of neutrophils is shown as relative number of ingested bacteria per cell, which correlates with the mean fluorescence intensity (MFI) of FITC. No difference between cells isolated by MACSxpress Technology or density gradient centrifugation was observed (n=6).

5 Neutrophils isolated by MACSxpress Technology are functionally active

Neutrophils were isolated from whole blood by MACSxpress Technology or density gradient centrifugation. Cells were incubated with E. coli and then analyzed by flow cytometry (Fig. 3). The MACSxpress Technology were analyzed for the expression of different activation and adhesion markers (Fig. 2). No difference in expression of apoptosis and cell death was found as well as Annexin V^+ and V^+ (Fig. 7).

6 MACSxpress Technology has no effect on viability of neutrophils

Neutrophils were isolated from whole blood by MACSxpress Technology and incubated with E. coli for 30 min (two ways: 2.1–3.3×10^6 cells per mL peripheral blood). Neutrophils isolated by MACSxpress Technology or density gradient centrifugation were stimulated with E. coli or PBS for 10 min at 37 °C (red line) for ROS induction (A). ROS production was analyzed by flow cytometry measuring the oxidation of the fluorogenic substrate DHR123. A sample without stimulus served as negative control (black line). (B) Frequency of ROS producing neutrophils with or without stimulus (n=6).

Conclusion

- MACSxpress Technology enables untouched isolation of neutrophils from whole blood in 20 min.
- No density gradient centrifugation required.
- High purity and high recovery of enriched neutrophils.
- No monocyte or eosinophil contamination of enriched neutrophils.
- Function of cells is unaffected.

- No density gradient centrifugation required.
- High purity and high recovery of enriched neutrophils.
- No monocyte or eosinophil contamination of enriched neutrophils.
- Function of cells is unaffected.