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

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Introduction

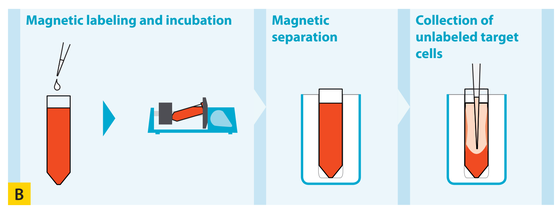
Human neutrophils are usually obtained by density gradient centrifugation. However, this method is exceedingly time-consuming and frequently results in neutrophil populations that are contaminated by other leukocytes, mainly eosinophils.

With the new MACSxpress Technology, untouched human neutrophils can be isolated from anticoagulated whole blood within 20 min. While erythrocytes are aggregated and sedimented, non-target cells are removed by immunomagnetic depletion with MACSxpress Beads, yielding untouched and functional target cells of high purity and recovery.

As cells from the first line 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 our 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. 3 and 4). In addition, neutrophils were tested for their phagocytic capacity by analyzing the uptake of FITC-labeled *E. coli* (fig. 5). 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 cytometric analysis of Annexin V showed that the isolation of neutrophils does not lead to increased induction of apoptosis (fig. 7)

Methods

Figure 1



The MACSxpress Neutrophil Isolation Kit enables the untouched isolation of human neutrophils from up to 8 mL anticoagulated 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 enriched target cells, is collected and transferred into a new tube. Magnetically labeled non-target cells as well as aggregated erythrocytes are retained in the tube (fig. 1B). The separation is fast, easy, and convenient. As the procedure does not require centrifugation, the formation of aerosols is avoided. This feature provides a high level of safety when working with untested blood samples.

Results

1 Neutrophils are efficiently enriched from whole blood by MACSxpress Technology

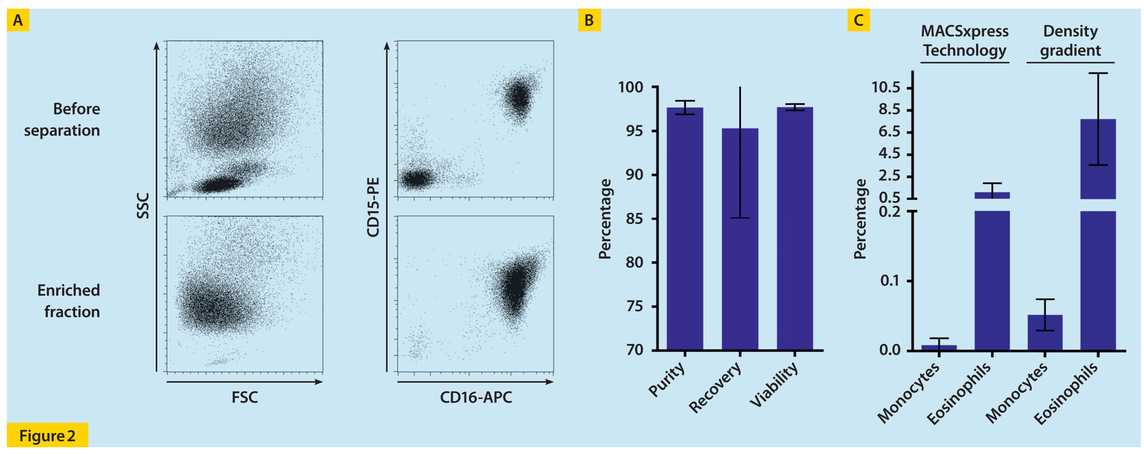


Figure 2

Purities, recoveries, and viabilities of neutrophils enriched from whole blood were assessed by flow cytometry using the MACSQuant Analyzer (fig. 2A,B). Neutrophils were defined as $CD15^+CD16^+$. Cell debris, non-leukocytes, and dead cells were excluded from the analysis based on CD45 expression, scatter signals, and propidium iodide (PI) fluorescence (fig. 2A). Figure 2B illustrates median purity (98±2%), recovery (95±23%), and viability (98±1%) of enriched neutrophils (n=6). The recovery correlated to the cell numbers of $2.1–3.3×10^6$ cells per mL peripheral blood. The target cell fraction contained only negligible numbers of monocytes and strongly reduced numbers of eosinophils compared to granulocytes isolated by density gradient centrifugation (fig. 2C). Eosinophils were defined as $CD15^+CD193^+$ cells and monocytes as $CD14^+CD56^-$ cells (n=6).

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

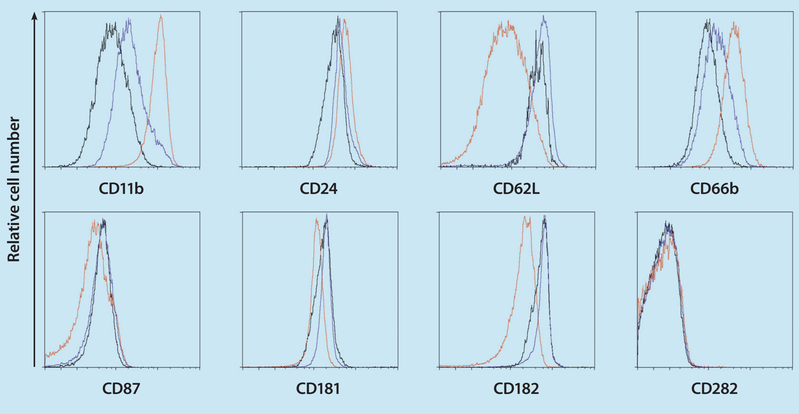


Figure 3

Peripheral blood cells (fig. 3, black line), neutrophils enriched by MACSxpress Technology (blue line), and cells isolated by density gradient centrifugation (red 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 $CD15^+CD16^+$ cells. CD11b, CD24, and CD66b expression was only 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 CD62L, CD181, CD182 and CD87 was reduced following density gradient centrifugation but not MACSxpress Enrichment, showing that adhesion/chemotactic and homing receptors were only slightly if at all influenced by the magnetic cell isolation process. Pattern recognition receptors CD282 and CD284 (not shown) were not influenced by MACSxpress Enrichment. One representative of three datasets is shown.

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

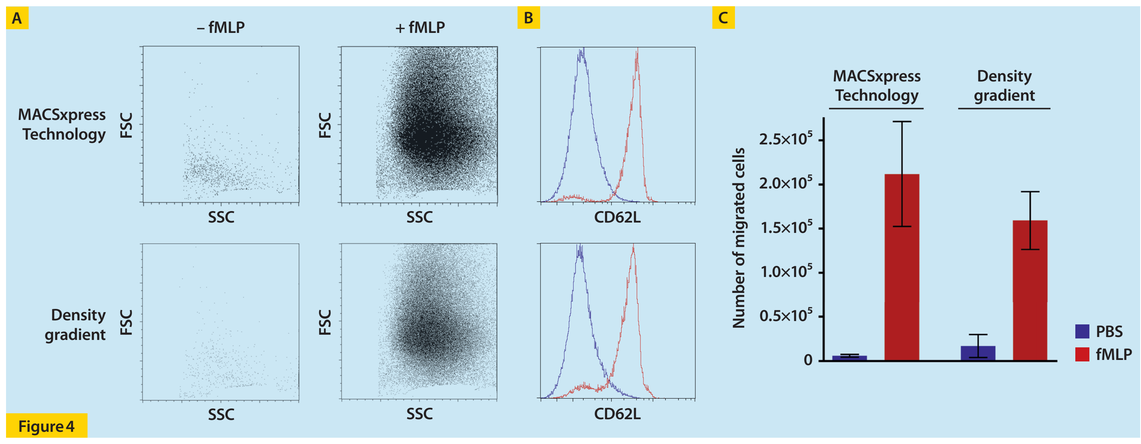


Figure 4

Chemotaxis of isolated neutrophils toward the peptide fMLP was analyzed using a transwell migration assay. Cells were allowed to migrate through the membranes for 30 min (pore size 3.0 µm). Transmigrated cells were quantified and analyzed by flow cytometry (fig. 4). (A) Shown are scatter characteristics of all neutrophils which transmigrated without stimulus or toward fMLP. (B) Adhesion molecule CD62L was shed from the surface of transmigrated (blue line), but not non-migrated neutrophils (red line). Results were independent of the cell isolation procedure. (C) Summary of migration experiments performed (n=8) showing the numbers of neutrophils which migrated without stimulus or toward fMLP respectively.

4 Isolation of neutrophils does not alter phagocytic capacity

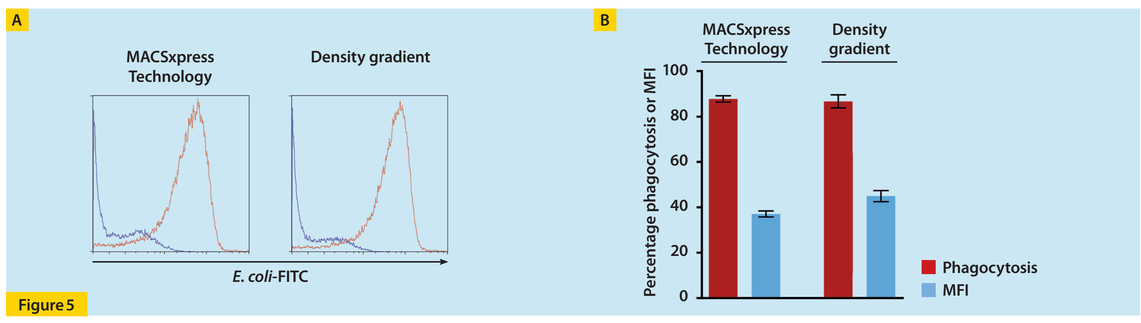


Figure 5

Neutrophils were isolated from whole blood by MACSxpress Technology or density gradient centrifugation. Cells were incubated with FITC-labeled immunoglobulin-opsonized *E. coli* either by 37 °C (red line) or on ice (blue line) for 10 min and then analyzed by flow cytometry (fig. 5A). Figure 5B shows the percentage of neutrophils which have 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

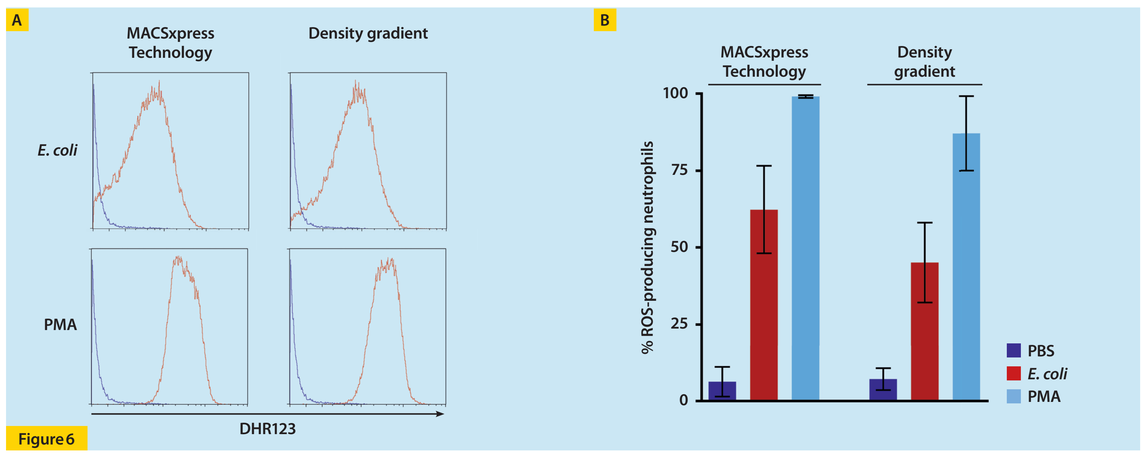


Figure 6

Neutrophils were analyzed for their capacity to produce ROS upon different stimuli (fig. 6). Cells isolated by MACSxpress Technology or density gradient centrifugation were stimulated with *E. coli* or PMA 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 (blue line). (B) Frequency of ROS-producing neutrophils with or without stimuli (n=8).

6 MACSxpress Technology has no effect on viability of neutrophils

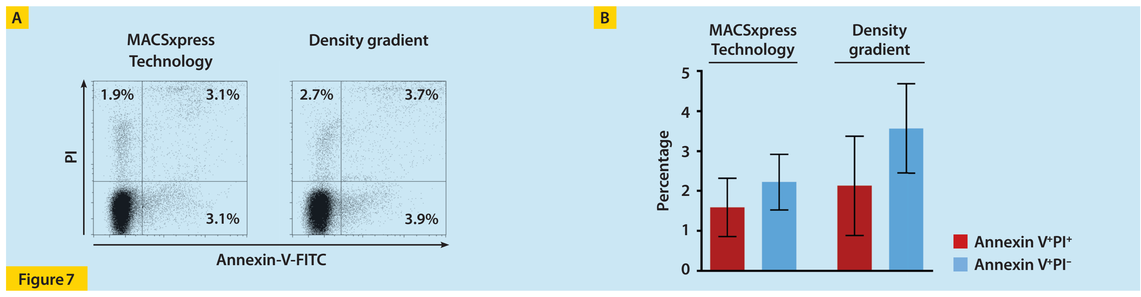


Figure 7

Enriched neutrophils were cultured for 3 h at 37 °C and then analyzed by flow cytometry for the expression of apoptotic markers (fig. 7). (A) Neutrophils undergoing early apoptosis were identified as Annexin V^+PI^- and dead cells/late apoptotic cells as Annexin V^+PI^+. (B) Frequencies of apoptotic and dead cells were not significantly influenced by the separation procedure (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.