Monocyte CD163 and CD36 Expression in Human Whole Blood and Isolated Mononuclear Cell Samples: Influence of Different Anticoagulants

Marcin Moniuszko,1* Krzysztof Kowal,1 Malgorzata Rusak,2 Miroslawa Pietruczuk,2 Milena Dabrowska,2 and Anna Bodzenta-Lukaszyk1

Department of Allergology and Internal Medicine, Medical University of Białystok, 15-276 Białystok, Poland,1 and Department of Hematological Diagnostics, Medical University of Białystok, 15-274 Białystok, Poland2

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We investigated whether the choice of anticoagulant or the application of density gradient mononuclear cell isolation may account for conflicting published data regarding the levels of the scavenger receptors’ expression in healthy individuals. We demonstrate that the detection of CD163, but not CD36, differs dramatically among the methods.

CD163 is a member of the scavenger receptor cysteine-rich family of proteins, accounting for the clearance of hemoglobin-haptoglobin complexes, which in turn fuel an anti-inflammatory response mediated by heme metabolites (6, 10). CD163 is also an attractive candidate for potential diagnostic use as a marker of monocyte/macrophage activity in inflammatory diseases (1, 3, 8, 11). However, it is still unclear how many circulating monocytes in normal subjects express the CD163 molecule on their surface. Previous studies with Mac 2-48, RM3/1, and other monoclonal antibodies indicated that 0 to 1% of monocytes were positive for CD163, as detected by flow cytometry (2, 4, 11, 12, 13). Possibly due to different protocols used for CD163 identification in various studies, some discrepancies were found even when the same clone of monoclonal antibody was used for different studies.

In the current study, we analyzed the influence of three commonly used anticoagulants and mononuclear cell isolation on the level of CD163 expression evaluated with the use of commercially available, fluorochrome-conjugated GHI/61 monoclonal antibody. For comparison, the same kind of analysis was performed for another scavenger receptor, CD36, which is also expressed on monocytes and is involved mainly in phagocytosis of apoptotic neutrophils and uptake of modified low-density lipoproteins.

Forty-eight individuals (20 female and 28 male) had blood drawn between 7 and 9 a.m. The donors were healthy volunteers aged 18 to 56 years and selected in accordance with the guidelines of the Białystok Local Ethics Committee (no. 8-I-003/132/2004). Blood samples were divided into tubes with EDTA, citrate, or heparin and immediately processed. Forty-eight individuals (20 female and 28 male) had blood drawn between 7 and 9 a.m. The donors were healthy volunteers aged 18 to 56 years and selected in accordance with the guidelines of the Białystok Local Ethics Committee (no. 8-I-003/132/2004). Blood samples were divided into tubes with EDTA, citrate, or heparin and immediately processed. Whole-blood samples were incubated with CD14 fluorescein isothiocyanate (clone M4P9), CD163 phycoerythrin (clone GHI/61), CD36 allophycocyanin (clone CB38) (all obtained from Becton Dickinson, San Jose, CA), and fluorochrome-conjugated isotype controls for 30 min at room temperature. Red blood cells were lysed for 10 min with fluorescence-activated cell sorter lysing solution (Becton Dickinson, San Jose, CA). The remaining white blood cells were washed twice with phosphate-buffered saline and fixed with 400 µl of 2% paraformaldehyde (5).

Portions of each whole-blood sample were also used for isolation of mononuclear cells. Mononuclear cells were isolated using Histopaque (density, 1.077 g/ml; Sigma-Aldrich) density gradient centrifugation as described previously (9). The isolated cells were washed twice with phosphate-buffered saline, and the cell concentration was adjusted to 1 × 10⁶ cells/ml. Mononuclear cells were stained with 20 µl of fluorochrome-conjugated monoclonal antibodies for 30 min at room temperature, washed twice with phosphate-buffered saline, and fixed with 2% paraformaldehyde.

A FACSCalibur cytometer (Becton Dickinson Immunocytometry Systems) was used to determine the surface intensity of various antigens on peripheral blood monocytes. In order to ensure the reproducibility of the data generated, the settings and calibration of the instrument fluorescence detectors were monitored and optimized on each day of analysis according to the manufacturer’s recommendations, using CaliBRITE beads (BDIS). The system linearity was evaluated using Spheroweb beads (Spherotech, Libertyville, IL). The coefficient of variation of all control beads used was less than 3% for scatter and fluorescence. Flow cytometry data were collected in list mode and analyzed using CellQuest software (BDIS).

The absolute numbers of monocytes of all donors were normal and ranged from 0.32 × 10⁹ to 0.69 × 10⁹ cells/liter. The CD14⁺ monocyte population was gated by a combination of forward and side scatter and CD14 gate sets. CD14⁺ monocytes constituted 75.6% to 89.2% and 64.3% to 78.7% of the entire monocyte population in whole-blood and mononuclear cell samples, respectively. To assess the expression levels of CD163 and CD36, the gate was initially set with corresponding isotype antibodies. The background values were 0.11% to 0.43% and 0.02% to 0.12% for phycoerythrin- and allophycocyanin-conjugated isotype antibodies, respectively.
The aim of the study was to evaluate the influence of various anticoagulants and density gradient mononuclear cell isolation on the detection of CD163 and CD36 on monocytes. We observed that CD163 assessed with the use of the GHI/61 anti-

sition was performed with logarithmic amplification. The data are presented as the percentages and geometric mean fluorescence intensities (MFI) of CD14⁺ monocytes coexpressing CD163 or CD36.

The flow cytometric data were analyzed for statistically significant differences between groups using Statistica software. The Mann-Whitney U test was applied, and statistically significant results were identified by a P value of <0.001.

Three widely used anticoagulants exerted various effects on CD163 monocyte expression (Fig. 1A). CD14⁺ monocytes in whole blood anticoagulated with EDTA presented with the highest expression of this molecule: 80.31% (13.33%) [mean (± standard deviation)] of them were positive for CD163 (MFI, 15.29 ± 3.61). When citrate was used for anticoagulation, the average level of expression of CD163 in the studied group was 65.4% (18.97%) of CD14⁺ monocytes (MFI, 11.6 ± 5.13) (P < 0.001 compared to the EDTA group). Interestingly, very little expression of CD163 (8.11% (23.51%) of CD14⁺ monocytes; MFI, 2.97 ± 0.59) was found on monocytes in freshly processed heparinized blood (P < 0.001 compared to either the EDTA or citrate group).

A different pattern of anticoagulant effect was observed during the analysis of CD36 expression (Fig. 1B). Although the highest levels of CD36 were observed on monocytes from citrated blood (96.9% ± 6.95% of CD14⁺ monocytes; MFI, 136.1 ± 24.11), similar values were also found for CD14⁺ monocytes from the EDTA and heparin samples (expression, 95.55% ± 4.78% and 95.39% ± 4.57%, respectively; MFI, 118.3 ± 22.88 and 119.1 ± 21.82, respectively). None of the differences among the anticoagulant groups was statistically significant.

Next, we evaluated the effects of the density gradient isolation on the monocyte surface marker expression. Again, the molecule most highly modulated by the procedure was CD163. After isolation, only 6.44% (18.34%) of CD14⁺ monocytes stained positive for CD163 (MFI, 4.31 ± 3.2) (P < 0.001 compared to the EDTA group). Interestingly, very little expression of CD163 (2.63% (3.32%) of CD14⁺ monocytes; MFI, 3.51 ± 0.53) was found on monocytes in freshly processed heparinized blood (P < 0.001 compared to either the EDTA or citrate group).

FIG. 1. CD163 and CD36 expression by CD14⁺ monocytes in whole-blood samples with regard to anticoagulant used. The results are the percentages of CD14⁺ monocytes positive for CD163 (A) and CD36 (B) (left panels) and the geometric MFI of the CD14⁺CD163⁺ (A) and CD14⁺CD36⁺ (B) monocytes (right panels), reported as the means ± standard deviations (n = 48). Statistically significant differences between the anticoagulant groups are indicated with stars (P < 0.001). PowerPoint 2003 and SigmaPlot 2000 software were used for creating this figure.
body was expressed with a significantly higher intensity on monocytes from samples anticoagulated with EDTA than on monocytes from the heparin and, to a lesser extent, citrate samples. A similar pattern of CD163 expression was also observed when the 5C6-FAT monoclonal antibody was used for staining (data not shown).

Regardless of the anticoagulant used, density gradient-isolated monocytes presented with very low levels of CD163 staining. Interestingly, in some of the previous reports, various portions of monocytes from isolated mononuclear cells were found to be positive for CD163 (2, 4, 12, 13; our unpublished data). The discrepancy with the results of our report may in part be explained by the fact that in the present study, we used GHI/61 monoclonal antibody, which is directed against a different domain of CD163 protein than those targeted by clones used in previous reports. This suggests that density gradient isolation may cause some conformational changes in the CD163 structure, which in turn prevent some monoclonal antibodies from binding to corresponding domains (7).

In summary, we propose the use of whole-blood samples rather than isolated mononuclear cells for direct ex vivo CD163 estimation with the use of GHI/61 antibody. Moreover, our results suggest that special care must be taken in choosing an anticoagulant for a sample in which CD163 is to be evaluated.

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