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

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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 Internal Medicine, Medical University of Bialystok, Sklodowskie-Curie Street 24A, 15-276 Bialystok, Poland. Phone: 48 509 138 579. Fax: 48 85 74 68 601. E-mail: moniuszm@amb.edu.pl.

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position was performed with logarithmic amplification. The data are presented as the percentages and geometric mean fluorescence intensities (MFI) of CD14\(^+\)/H11001 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\% (MFI, 4.31 ± 3.2) of CD14\(^+\) monocytes stained positive for CD163. A similar effect of density gradient isolation was seen in mononuclear cells from citrated and heparinized blood: only 2.63\% ± 1.81\% (MFI, 3.51 ± 0.53) and 2.26\% ± 3.32\% (MFI, 3.18 ± 0.66) of CD14\(^+\) monocytes were positive for CD163, respectively (Fig. 2A). Interestingly, mononuclear cell isolation did not alter the percentage of monocytes positive for CD36 compared to what was seen with whole-blood samples (Fig. 2B). In the case of peripheral blood mononuclear cells, none of the differences among the anticoagulant groups for both CD163 and CD36 was statistically significant.

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-

![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.](http://cvi.asm.org/)
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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FIG. 2. Summary of analyses of CD163 (A) and CD36 (B) staining on the surface of CD14+ monocytes in isolated peripheral blood mononuclear cell samples with regard to anticoagulant used. Cells were analyzed as described in the legend to Fig. 1. PowerPoint 2003 and SigmaPlot 2000 software were used for creating this figure.
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