



Hospices Civils de Lyon



Monitoring of sepsis-induced monocytic HLA-DR expression and regulatory T cells alterations



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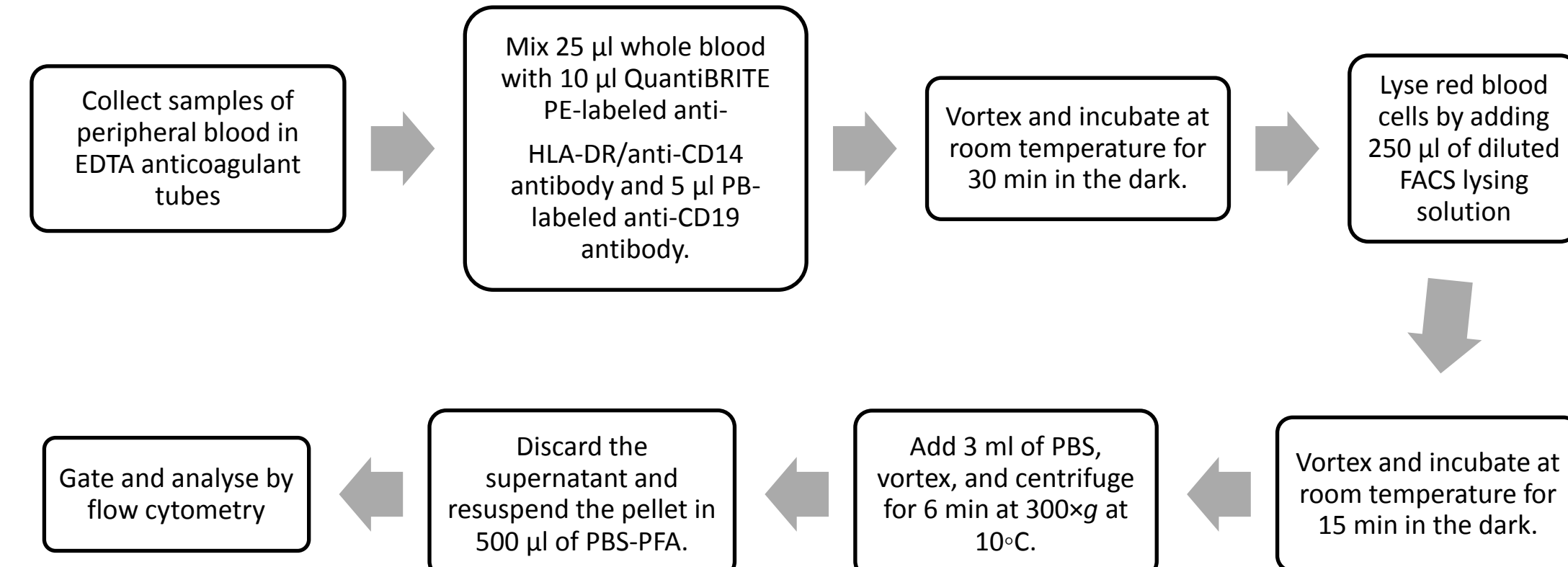
Introduction

Sepsis (i.e. multiple-organ dysfunction associated with a dysregulated host response to infection) is the leading cause of death in noncoronary intensive care unit and represents a major, although largely under-recognized, health care problem. It is especially common in the elderly and continues to increase substantially as the population ages. In sepsis, multiple pro- and anti-inflammatory responses initiated by an invading pathogen are mounted simultaneously and can evolve to an exaggerated inflammation and immunosuppression. Currently, diagnosis of immunosuppression relies on nonspecific physiological criteria and lacks specificity. As a result, most sepsis trials have failed to show a benefit of newly developed immunomodulatory therapies and severe sepsis remains associated with a high mortality rate. It is therefore vital to develop reliable and effective biomarkers for the diagnosis of sepsis. To date, trials have shown that monocytic HLA-DR is a good marker for monitoring the severity of immunodepression. Also, CD4⁺CD25⁺CD127_{low} regulatory T cell percentage may represent a reliable marker for the diagnosis of lymphocyte dysfunctions after sepsis. This review reports on the immune alterations in these biomarkers during sepsis. The objective is to investigate an up-to-date account of clinical results for HLA-DR and Treg assays.

Patients and Methods

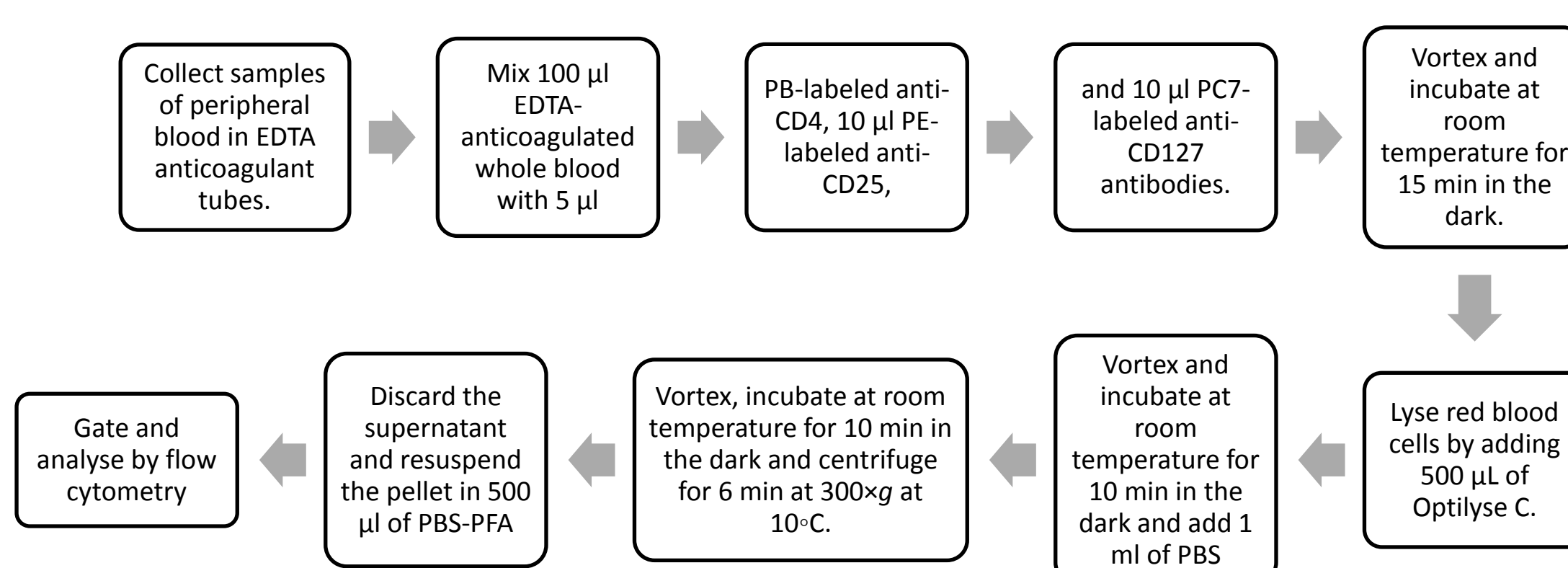
mHLA-DR Expression

Peripheral blood was collected from 45 patients at day 1 or 2 (D1-2), 3 or 4 (D3-4) and between day 6 and 8 after sepsis diagnosis, as well as at the day of discharge (DD). In addition, 45 healthy volunteers (HV) donors provided control samples. The mean of fluorescence intensity (MFI) was measured by flow cytometry. QuantiBRITE™PE beads were used to convert MFI in numbers of bound anti-HLA-DR antibody (AB/c) per monocyte. Differences between groups were tested using the non-parametric Mann-Whitney test with Prism software (*: $p < 0.05$ **: $p < 0.01$ ***: $p < 0.001$).



Regulatory T Cells

The blood samples were obtained from the same 45 patients but compared with reference values and collected at day 3 or 4 (D3-4) after sepsis diagnosis and at the day of discharge (DD). Absolute count of CD4⁺ measurements were performed using Flow-count fluorospheres™. Group differences were tested for significance using the Wilcoxon signed-rank test with Prism software (*: $p < 0.05$ **: $p < 0.01$ ***: $p < 0.001$).



Results

mHLA-DR Expression

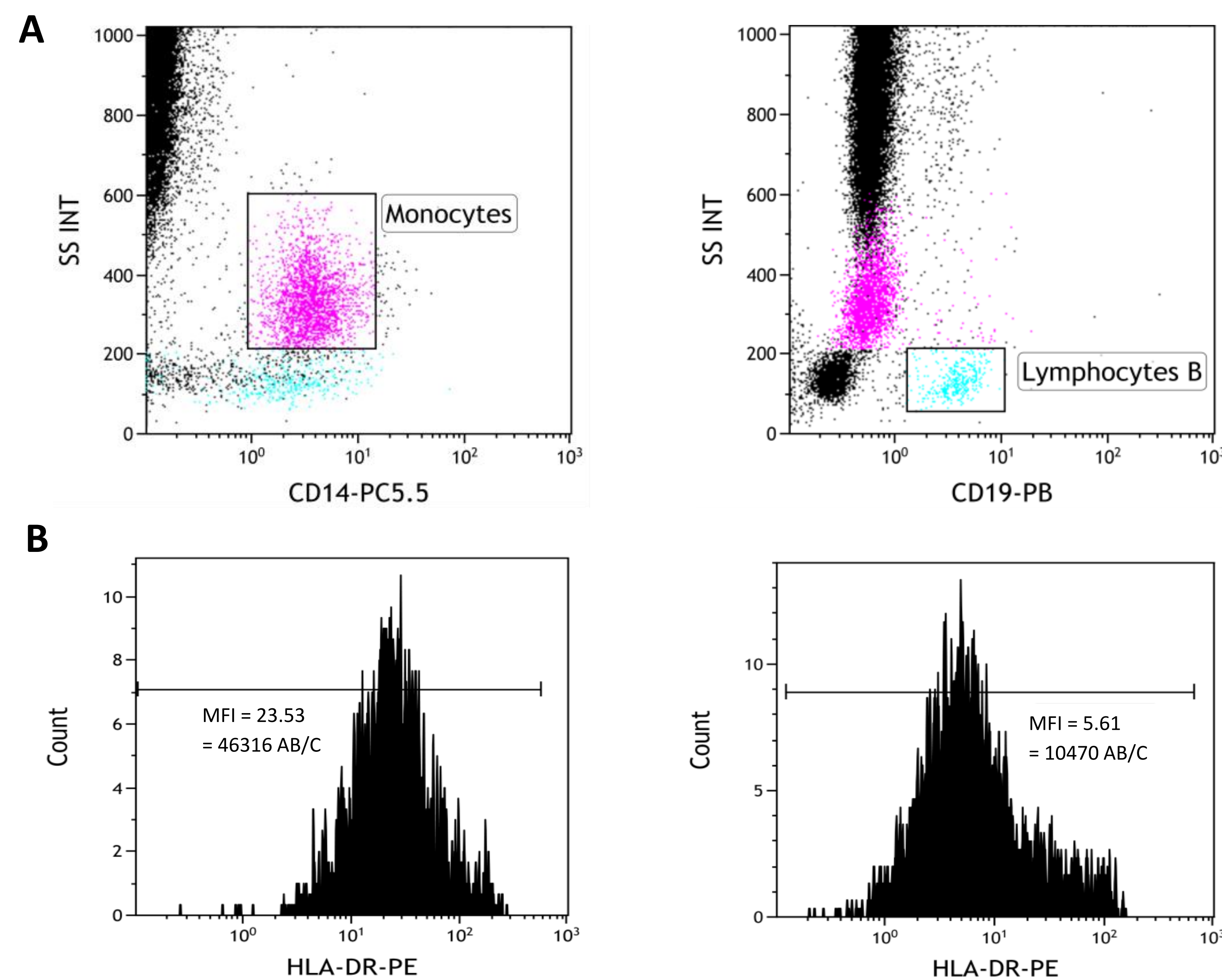


Figure 1. Monocyte HLA-DR expression measurement by flow cytometry. (a) Representative CD14 versus side scatter (SS) dot-plot that allows for the gating of CD14⁺ monocytes. **(b)** Representative HLA-DR linear histograms gated on monocytes in a healthy volunteer and a septic patient. (PE, phycoerythrin)

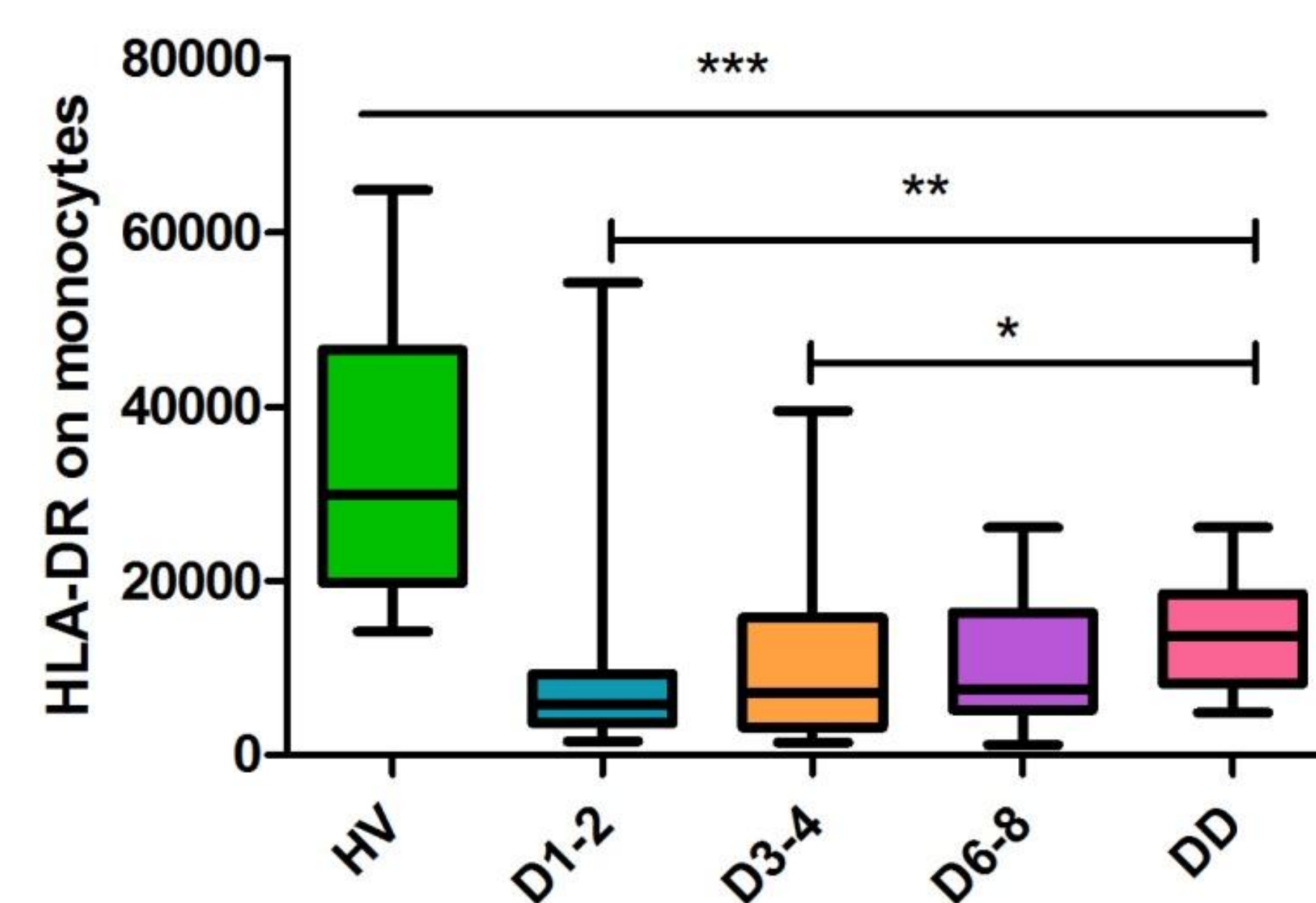


Figure 2. Monocyte HLA-DR expression in septic patients and healthy volunteers. Results are expressed as numbers of anti-HLA-DR antibodies bound per cells (AB/c) of whole blood sampled at D1-2 (n= 36), D3-4 (n= 38), D6-8 (n= 21) and DD (n= 16).

Regulatory T Cells

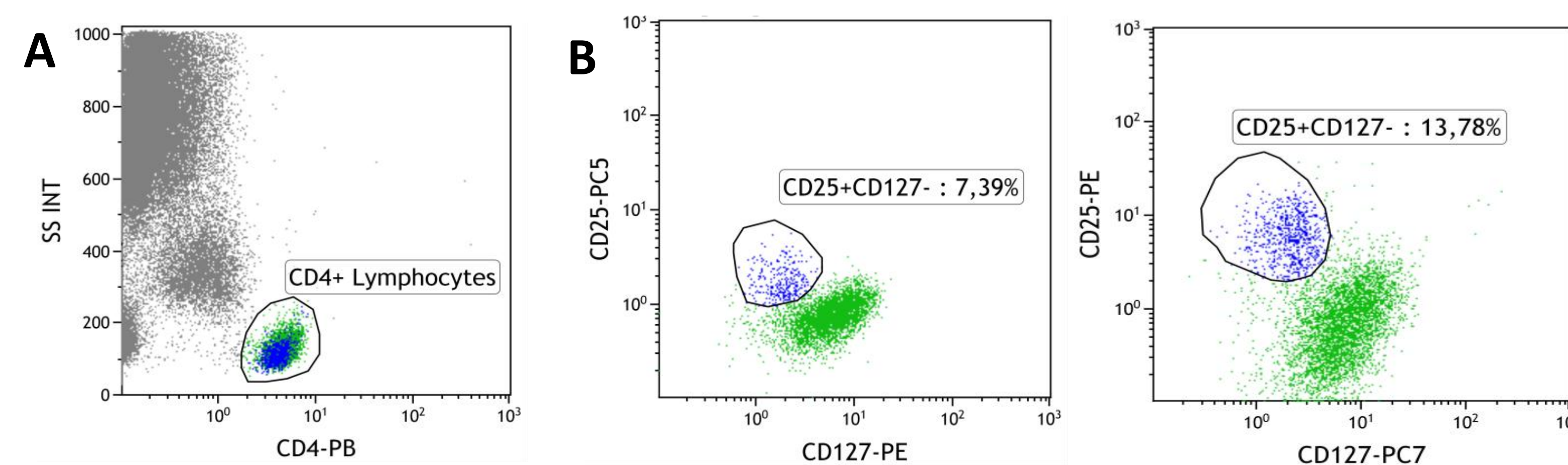


Figure 3. CD4⁺CD25⁺CD127_{low} regulatory T cell measurement by flow cytometry. (a) Representative CD4 versus side scatter (SS) dot-plot that allows for the gating of CD4⁺ lymphocytes. **(b)** Gated on CD4⁺ Lymphocytes, representative CD25 versus CD127 dot-plots in one healthy volunteer and a septic patient. The CD25⁺CD127_{low} population is easily identified and its percentage is slightly increased in septic patients. (PB, pacific blue; PE, phycoerythrin; PC7, phycoerythrin-cyanine 7)

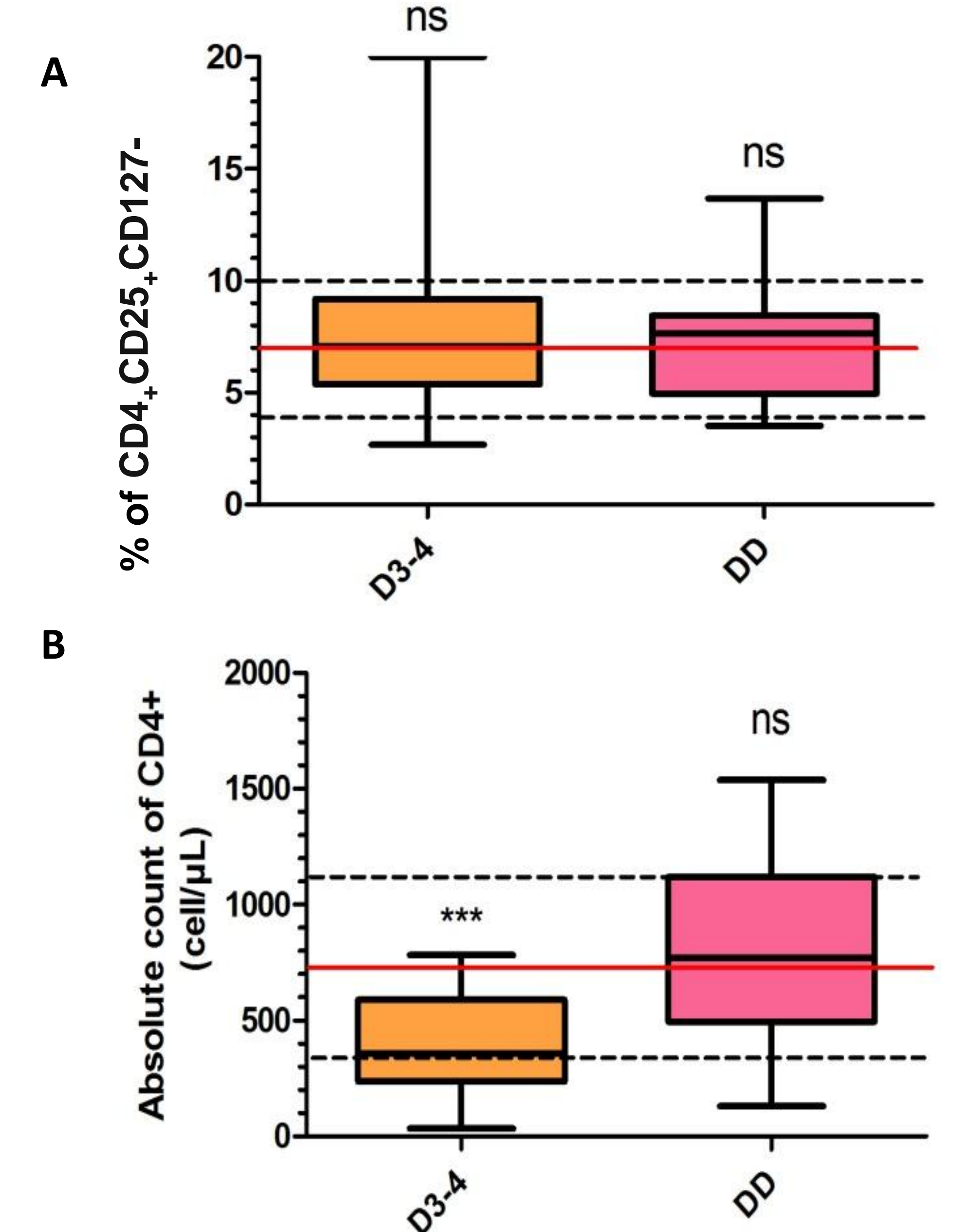


Figure 4. Percentage of CD4⁺CD25⁺CD127_{low} regulatory T cell and number of CD4⁺ T lymphocytes in septic patients. The dotted lines represent the lower and upper limits of the reference range and the red line is the mean value. **(a)** Percentage of CD4⁺CD25⁺CD127_{low} lymphocytes measured among CD4⁺ cells at D3-4 (n= 37) and DD (n= 13). **(b)** Absolute count of peripheral CD4⁺ T lymphocytes of whole blood at D3-4 (n= 37) and DD (n= 13).

Conclusion

A decreased cell surface expression of HLA-DR was observed on circulating monocytes (mHLA-DR) in septic patients at D1 to DD. This suggests decreased mHLA-DR is a reliable marker for the diagnosis of immunosuppression and predictive of adverse outcome in critically ill patients.

No significant increase in the percentage of circulating CD4⁺CD25⁺CD127_{low} Treg in septic patients was observed in comparison with the standard reference values. Thus, the relative increase observed is not enough to represent a standardizable marker of declining proliferative capacity after sepsis.

Ideally, large multicenter standardized studies investigating various and more detailed aspects of immune response after sepsis would be of major interest to test individualized immunotherapy.

References and Acknowledgments

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References available upon request.