Optimization of Antibody Reduction Conditions for Improvement of Conjugation to Fluorochromes

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Introduction: The Brazilian Health Ministry demands constant evaluation of lymphocytes CD4+ and CD8+ levels on HIV serum positive patients by Flow Cytometry assay. In this methodology, monoclonal immunoglobulins anti-CD45, anti-CD3, anti-CD4 and anti-CD8 are labeled with fluorochromes, such as fluorescein, phycobiliproteins (PE, PECy7, APC) or cyanines. IgG and phycobiliproteins can be conjugated using free thiol groups generated by IgG partial reduction.

Material and Methods: The fluorochrome Phycoerythrin (PE) was selected for conjugation with anti-CD8. In an attempt to improve the yield of the conjugation process, we have optimized the classical Roederer’s protocol. The monoclonal antibody anti-CD8 preparation was quantified by absorbance at 280nm and its homogeneity was verified by PAGE discontinuous, IEF-PAGE and SDS-PAGE. Anti-CD8 was reduced by DTT or β-mercaptoethanol at different concentrations (0.15 – 200 mM) and reaction times (1- 60 min). The reduced IgG was analyzed by SDS-PAGE.

Results and Discussion: The anti-CD8 preparation was homogeneous by electrophoretic analysis. Both DTT and β-mercaptoethanol were able to partially reduce anti-CD8, as observed by SDS-PAGE. Taking into account that IgGs are tetramers formed by two heavy chains (H) and two light chains (L), the theoretical partial reduction would produce HHL, HH, HL and L forms. The major forms observed for us were HHL, HH and L. The optimized reduction condition using DTT 0.15 mM for 5 minutes was selected because favours the formation of HHL molecule, the only appropriated IgG form, among the observed ones, for production of conjugates to the immunophenotyping assay by flow citometry.

Conclusions: The classical Roederer’s reduction protocol of the monoclonal anti-CD8 preferentially produced the HH molecule. The optimized reduction condition improved the formation of HHL, the recommended IgG form for fluorochrome conjugation aiming flow citometry analysis.

Keywords: antibody reduction, flow citometry, immunophenotyping assay.

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