R7 Comparative study of physico-chemical and immunological properties of the monoclonal anti-CD4 produced by bioreactor and murine sources

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Introduction: The Institute of Technology in Immunobiology Bio-Manguinhos has been developing a kit of immunophenotyping to quantify CD4 + and CD8 + lymphocytes levels on HIV serum positive patients by flow cytometry. Currently, this kit consists of monoclonal antibodies (anti-CD3, anti-CD4, anti-CD8 and anti-CD45) produced in mice by the Center of Molecular Immunology (CIM) in Cuba. Aiming to improve the antibodies production by application of the 3R`s rule (**R**eduction the number of animals, **R**efinement of production conditions and **R**eplacement of *in vivo* assays), bioreactor production has been employed through scientific cooperation between
CIM and Bio-Manguinhos.

Objective: In this work we have compared the monoclonal anti-CD4 from bioreactor process to that produced by murine, to evaluate if the first one can be used to compose the immunophenotyping kit.

Methodology: Monoclonal antibodies anti-CD4 were analysed by Size Exclusion Chromatography (SEC) on Superdex 200 column 10/300, gradient SDS-PAGE (8 – 25%) and native IEF-PAGE (3.0 – 9.0). Tryptophan fluorescence emission spectra were obtained by setting the excitation wavelength at 280 nm, and the emission spectrum was recorded from 295 to 415 nm and Circular Dichroism (CD spectra were monitored from 200 to 260 nm. At last, the anti-CD4 was conjugated to fluorocrome PE to be immunologically evaluated by flow cytometry (FC).

Results: The SEC analysis of monoclonal anti-CD4 produced by bioreactor and murine showed similar chromatography profile presenting a single homogeneous peak. The anti-CD4 from murine and bioreactor presented by denaturing gel electrophoresis two bands with MW compatible with light and heavy chains of gamma immunoglobulin and relative standard deviation around 0.15%. Slightly differences in the distribution pattern of the multiple bands were observed at pH range of 7.0-6.30 by isoelectric focusing, probably it is due to carbohydrate microheterogeneity. CD and fluorescence spectra were identical for the antibodies analyzed, suggesting that they present the same conformational structure and reinforcing the hypothesis of carbohydrate microheterogeneity. The anti-CD4 antibodies presented the same profile and titer by flow cytometry analysis of +CD4 lymphocytes.

Conclusion: Based on the methods used so far the anti-CD4 from bioreactor is equivalent to that produced by murine and can be utilized in the immunophenotyping kit. More experiments such as N-terminal amino acid sequencing and/or mass spectrometry could be used to enclose this study.

Keywords: Monoclonal Antibody, +CD4 lymphocytes