FLOW CYTOMETRY STUDIES OF THE HIV-ENVELOPE MEDIATED CELL-CELL FUSION

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Syncytia formation is considered a potential cell depleting mechanism in the HIV-infection. The extent of fusion and cell composition of syncytia may be related to their biological significance and eventual fate. We developed an approach for the study of the HIV-envelope mediated cell-cell fusion that integrates the study of the fusion quantitative parameters (number and diversity of syncytia), with functional considerations and the cell population dynamics during fusion. A fusion assay was designed which is based on the staining of fusion partners with either lipophilic or cytoplasmic fluorescent dyes, followed by flow cytometry analysis. Syncytia and cells remaining unfused can be monitored as double and single colored particles, respectively. Fluorescence resonance energy transfer (FRET) between dyes allows to discriminate fused cells from cellular aggregates. The number, size and cellular composition of syncytia varied with time of reaction, coculture ratio and with Env-expression levels, indicating the formation of quantitative and qualitative different syncytia. Syncytia formation associated with a great reduction of the number of surrounding single cells, which also showed a low expression of receptors important for the immune function. Serum antibodies from HIV-patients inhibited or enhanced fusion in a way related with patient's viral load and clinical status. These observations indicate a significant role of cell-cell fusion in AIDS-pathogenesis. Besides the excellent performance of the flow cytometry assay in the biological characterization of syncytia formation, it is valuable for the screening of compounds with potential as inhibitors or enhancers of the HIV-envelope dependent cell-cell fusion.

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