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Determination of intracellular P19 core protein in HTLV-I infected cells by flow microcytofluorimetry analysis

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INTRODUCTION

HTLV-I is a human retrovirus linked to Adult T cell leukemia and to cases of cutaneous T cell lymphoma [1,2].

The preferential target of HTLV-I infection is the CD4+T cell subset. Moreover, HTLV-I is able to immortalize a broad range of lymphoid and non lymphoid cells *in vitro*. Particularly, mononuclear cells from cord blood are more susceptible to HTLV-I infection than those obtained from peripheral blood of adult donors [3].

The experimental model of *in vitro* HTLV-I transmission, is based mainly on cocultures between inactivated (i.e. irradiated) HTLV-I donor cells and recipient cells from different sources [4].

Virus infection is then monitored through molecular studies, in order to investigate the presence of HTLV-I DNA provirus and RNA transcripts within the infected cells [5]. Virus transmission to recipient cells can be also monitored by the expression of virus core protein p19, determined by an indirect immunofluorescence assay (IFA) [6].

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In order to semplify and improve HTLV-I p19 protein evaluation, experiments were carried out using flow microcytofluorometry (FMC) analysis to replace conventional fluorescence microscopy. Moreover FMC analysis provides detailed information on infected cells, inasmuch the samples can be examined using additional parameters other than fluorescence such as volume and light scatter or more than 5000 events. This study was initially approached using the chronically infected HTLV-I cell line MT-2. Usually MT-2 cells are 95-99% positive for the virus core protein p19, as determinated by IFA at fluorescence microscope.

MATERIALS AND METHODS

Cell cultures

The MT-2 line, is a HTLV-I producer T cell line derived from human cord blood mononuclear cells infected with the virus following cocultivation with human leukemic T cells [4]. MT-2 cells were maintained in RPMI-1640 medium (GIBCO, Paisley, Scotland, UK) supplemented with 20% foetal calf serum (GIBCO), 2mM L-glutamine (Flow Laboratories, McLean, VA, USA) and 2mM L-glutamine (Flow Laboratories, McLean, VA, USA) and antibiotics.

Fixation

The cells were washed in phosphate buffered saline (PBS), pH 7.5, and fixed either in ethanol 50-70% or in methanol 95% for 5- 10 min. at 4°C [7].

Immunofluorescence staining and FMC analysis

MT-2 cells were incubated with 1:400 dilution of anti-p19 monoclonal antibody (mAb) [6], kindly provided by Dr. M. Robert- Guroff (National Cancer Institute, NIH, Bethesda, MD, USA) for 30 min. at 4°C. Cells were then washed and incubated with fluorescein isothiocyanate (FITC)-conjugated $F(ab')^2$ goat anti-mouse IgG for 30 min. at 4°C. Negative controls were set up by incubating cells with FITC-conjugated $F(ab')_2$ goat anti-mouse IgG only. After, two washes in PBS the samples were run at the flow cytometry (FACScan, Becton Dickinson, Mountain View, CA, USA) and analyzed by the appropriate software (FACSCan).

RESULTS

FMC allows the gating of cell populations to be analyzed on the basis of volume and light scatter parameters. The mean fluorescence value and the percentage of positive cells of each sample are illustrated by an histogram. Conventional microscopic evaluation of p19 positivity of MT-2 cells used for FMC analysis, showed a range of 95-97% positive cells in 6 different tests (data not shown). The same samples were subjected to FMC analysis, and the results, illustrated in *Figure 1*, show the fluorescence distribution of the negative control (*Figure 1A*), of MT-2 cells labeled with the anti-p19 mAb (*Figure 1B*), and the comparison between the two curves (*Figure 1C*). The range of p19 positive cells in MT-2 line was found to be from 90 to 95%. Fixed MT-2 line shows two cell populations characterized by different size, that can be gated and analyzed separately. «Large size» cells are highly positive for the virus core protein p19 but are also remarkably autofluorescent (*Figure 2A,C*). On the other side fluorescence distribution histogram for gated«small size» cells

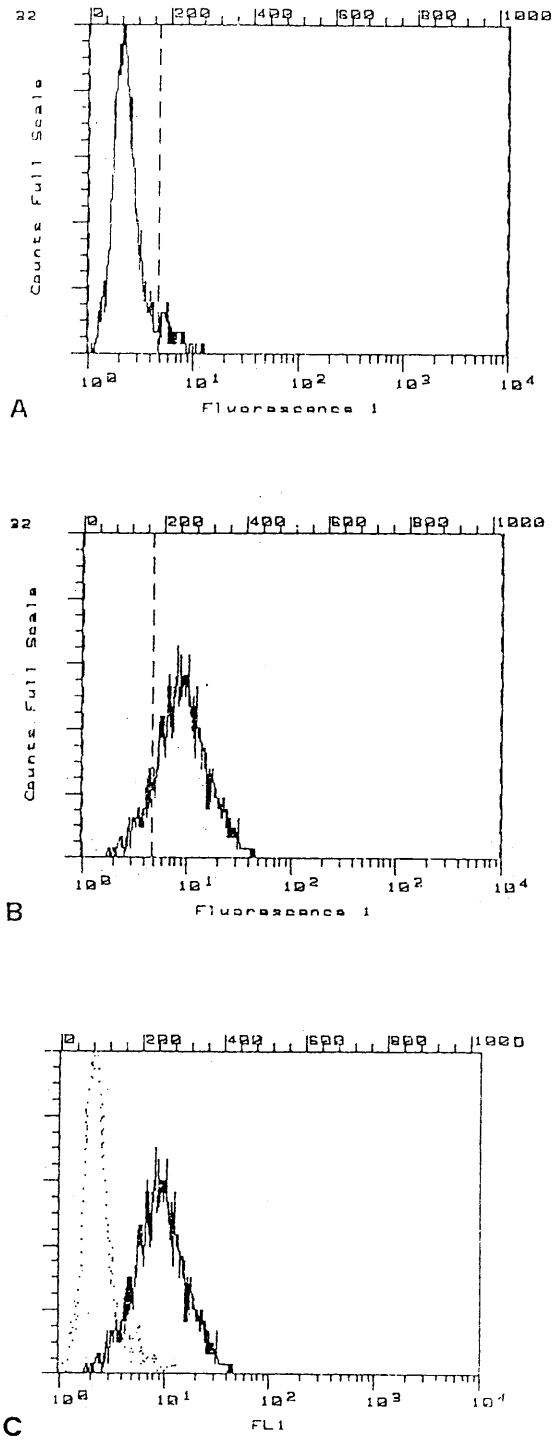

Figure 1 - P19 expression of MT-2 by FMC analysis. A) MT-2 incubated with FITC-labeled $F(ab')_2$ goat anti-mouse IgG. B) MT-2 labeled with anti-p19 mAb and FITC-labeled $F(ab')_2$ goat anti-mouse IgG. C) Comparison between the two curves, according to«merge» program.

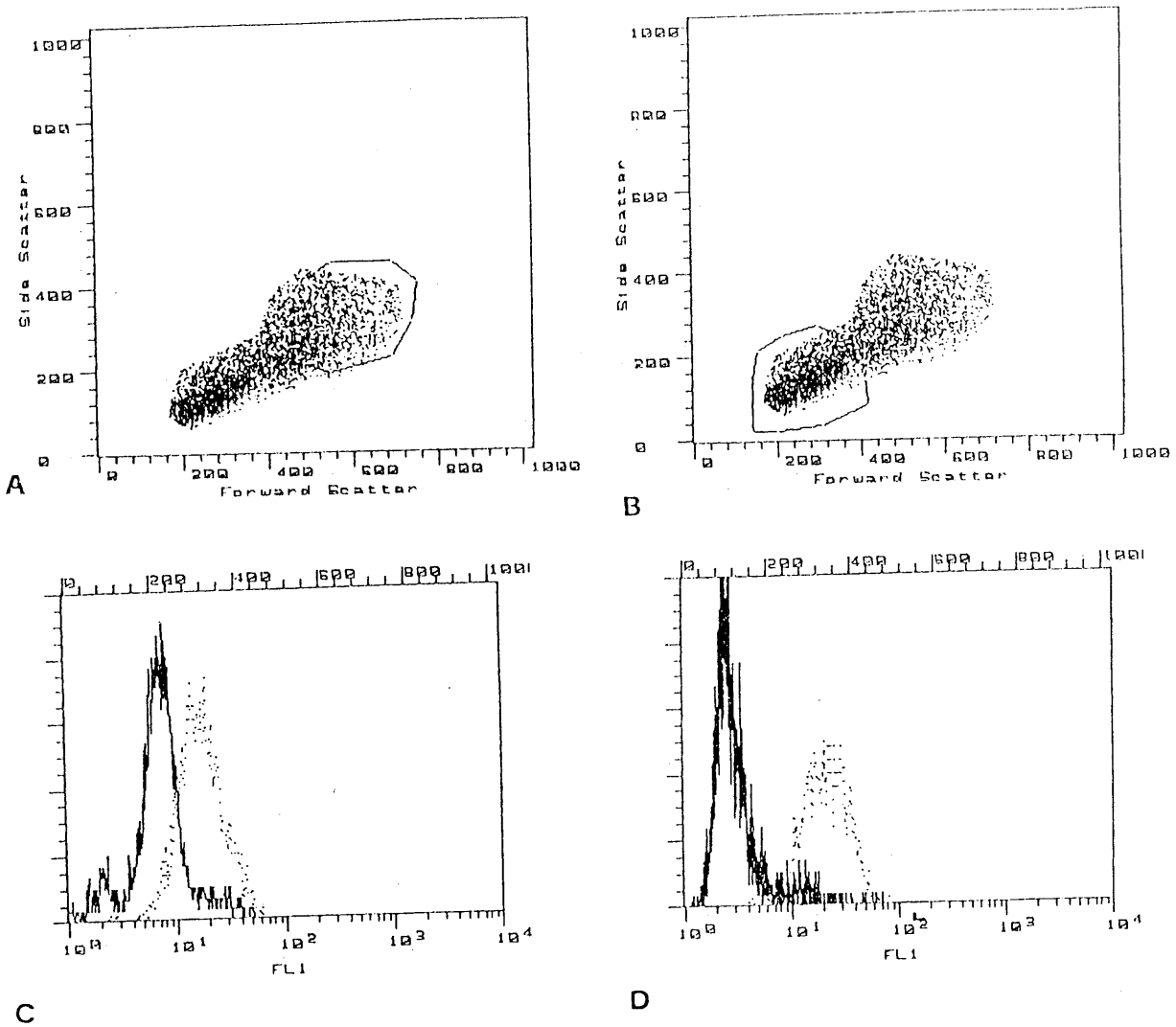

Figure 2 - Gate analysis of two MT-2 cell populations, following fixation. A) MT-2 cells with large size. B) MT-2 cells with small size. C) Fluorescence histograms of large size p19 cells: (----) Negative Control; (....) MT-2 cells labeled with anti-p19 mAb. D) Fluorescence histograms of small size p19 cells: (----) Negative Control; (....) MT-2 cells labeled with anti-p19 mAb. The data were analyzed by «merge program».

stained for p19 antigen shows only marginal overlap with that of gated negative control (*Figure 2B,D*). Further studies were performed to test wether the percentage of p19 positive cells evaluated by FMC analysis were consistent with the actual number of cells labeled with anti-p19 mAb. Unlabeled MT-2 cells were admixed with graded numbers of fluorescent cells of the same line, and analyzed by FMC. The results (*Figure 3*) show that the percentage of p19 positive cells detectable by FMC analysis is consistent with the actual percentage of the added cells (i.e. 80, 40 and 20%).

DISCUSSION

The preliminary data presented in this report suggest that FMC analysis can be applied to the determination of viral intracellular antigens in HTLV-I infected cell lines. The advantages of this technique can be summarized as pfollows:

a) the number of events that can be considered are 5,000 or more, thus determining the percentage of positive cells with great accuracy;

b) FMC analysis through the mean fluorescence value of each sample, would provide an

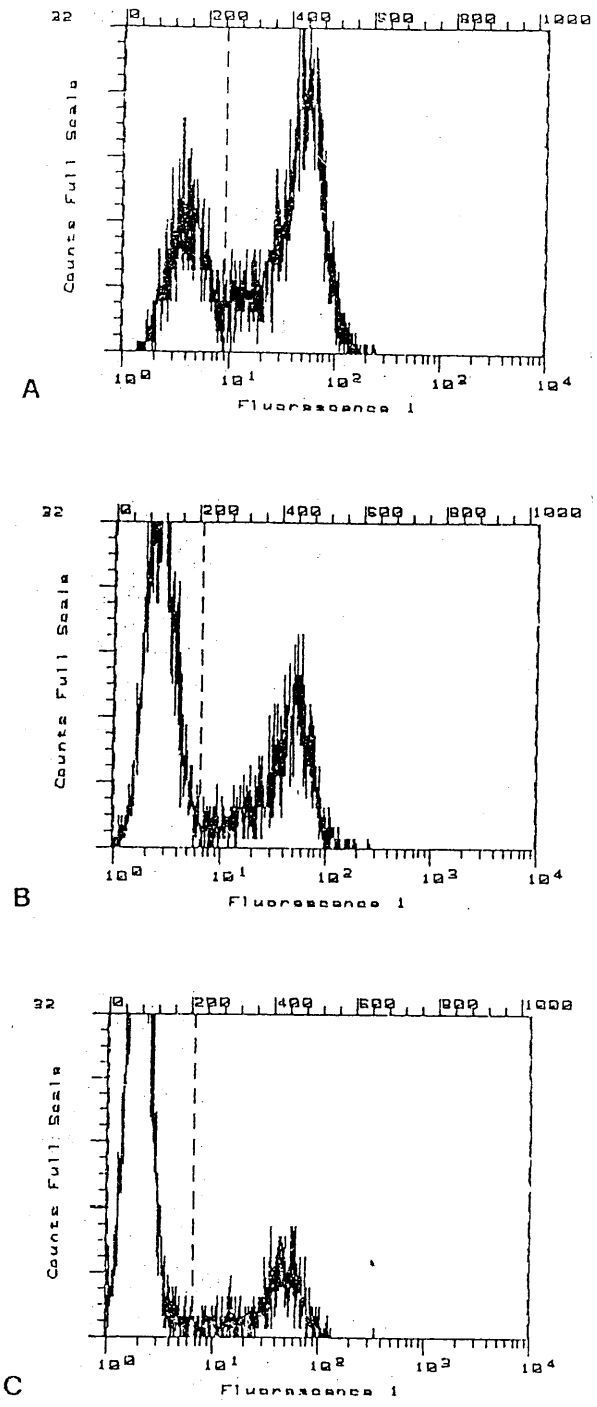

Figure 3 - FMC analysis of MT-2 cell samples set up by addding a prefixed percentage of MT-2 fluorescent cells. A) 80% of MT-2 fluorescent cells; B) 40% of MT-2 fluorescent cells; C) 20% of MT-2 fluorescent cells. The histogram on the left side of the marker (set by running MT-2 cells labeled with FITC-conjugated goat anti-mouse IgG only) shows the percentage of non fluorescent cells in each sample.

estimate of the amount of viral antigens expressed by the cells;

c) the setting up of gates eliminates the possibility to include debris or dead cells in the analysis of the samples. However, it has to be taken into consideration that fixed cells can change their morphology, and this volume variation has to be considered in the FMC analysis.

Future developments of this method open up the possibility to identify the virus-producing cells through a double labeling of both intracellular viral antigens and membrane antigenic markers of acceptor cells. In addition, the present method could provide a quick procedure to investigate the kinetics of possible protective effects of antiviral and/or immunomodulant agents on HTLV-I infection *in vitro*.

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