Abstract:
Tracking deuterium ((2)H) incorporation into cellular DNA, after administration of (2)H(2)O or (2)H(2)-glucose, is a recently developed, broadly applicable method for measuring in vivo cell proliferation and turnover that can be used safely in humans. This approach has been used to evaluate the turnover of T-cell subpopulations purified from the peripheral blood of HIV-1-infected patients using fluorescence-activated cell sorting (FACS). A requirement for widespread adoption of this approach for medical decision-making and for use in larger clinical trials is a simple, reproducible, high-throughput method for isolation of highly purified CD4(+) T cells from peripheral blood. Here, we present a simple method, which does not require FACS, for isolating these cells in sufficient purity and yield for analysis of (2)H incorporation into DNA. When blood from HIV-1-infected patients was used, neither the depletion of unwanted cell lineages by erythrocyte crosslinking (RosetteSep) nor the enrichment of CD4(+) cells by immunomagnetic beads (MACS) individually resulted in sufficient purity. The successive application of the two techniques, however, permitted isolation of >95% pure CD4(+) T cells in adequate yield (>10(6) cells/10 ml blood) from healthy donors and HIV-1-infected patients with CD4 counts between 300 and 700 cells/microl. Moreover, (2)H incorporation into cellular DNA after
administration of (2)H(2)O to HIV-1-infected patients was indistinguishable between CD4(+) T cells isolated by RosetteSep/MACS and FACS. Thus, both FACS and the new method isolate a similar mixture of long- and short-lived CD4(+) T cells. In practice, the RosetteSep/MACS method is simple, rapid, robust and capable of high throughput.

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