IL-7 differentially regulates cell cycle progression and HIV-1-based vector infection in neonatal and adult CD4$^+$ T cells

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Differences in the immunological reactivity of umbilical cord (UC) and adult peripheral blood (APB) T cells are poorly understood. Here, we show that IL-7, a cytokine involved in lymphoid homeostasis, has distinct regulatory effects on APB and UC lymphocytes. Neither naive nor memory APB CD4$^+$ cells proliferated in response to IL-7, whereas naive UC CD4$^+$ lymphocytes underwent multiple divisions. Nevertheless, both naive and memory IL-7-treated APB T cells progressed into the G_{1b} phase of the cell cycle, albeit at higher levels in the latter subset. The IL-7-treated memory CD4$^+$ lymphocyte population was significantly more susceptible to infection with an HIV-1-derived vector than dividing CD4$^+$ UC lymphocytes. However, activation through the T cell receptor rendered UC lymphocytes fully susceptible to HIV-1-based vector infection. These data unveil differences between UC and APB CD4$^+$ T cells with regard to IL-7-mediated cell cycle progression and HIV-1-based vector infectivity. This evidence indicates that IL-7 differentially regulates lymphoid homeostasis in adults and neonates.

Naive and memory T lymphocytes enable the immune system to maintain reactivity to new antigens and generate vigorous recall responses, respectively. Although the adult lymphocyte compartment is composed of approximately equivalent proportions of naive and memory T cells, neonatal lymphocytes are distinct in that they are almost entirely naive. It is significant, however, that the factors controlling the extrathymic expansion and maintenance of the naive T cell pool in humans are poorly understood. Several lines of evidence suggest that, at least in mice, the mechanisms that maintain the naive and memory T cell compartment differ. Mouse memory T cells can survive for up to 18 months in the absence of any interaction with autologous major histocompatibility complex molecules, whereas the life span of naive murine T cells is considerably shorter (1–9). Additionally, although several studies have shown a role for cytokines in signaling through the common gamma chain (γ_c), IL-2, IL-4, IL-7, and IL-15, in the proliferation of CD4$^+$ and CD8$^+$ murine lymphocytes (6, 10–12), it is unlikely that the same cytokines regulate the survival and proliferation of naive and memory T cells. Indeed, it has recently been demonstrated that naive T cells cannot survive in mice lacking the γ_c chain, whereas memory T cell survival is not restricted in this manner (13). More specifically, the combination of IL-4 and IL-7 contributes to naive, but not memory, murine CD4$^+$ T cell survival (10), and IL-7 mediates the proliferation of naive T cells in lymphopenic mice (14).

In humans, a role for IL-7 in extrathymic lymphocyte maintenance is suggested by recent data demonstrating that this cytokine functions as an *in vitro* survival factor for naive T cells isolated from neonates [umbilical cord (UC)] and adults [adult peripheral blood (APB)] (15–17). IL-7 does not alter the phenotype of naive lymphocytes, and activation markers are not induced (15, 17–19). IL-7 has potentiated the effects of T cell receptor (TCR) stimulation on human CD4$^+$ T cell proliferation (16, 17), but it is not known whether IL-7 itself induces lymphocyte expansion. Additionally, it is not clear whether naive UC lymphocytes and naive APB lymphocytes respond equivalently to IL-7. This question is raised by recent data suggesting that UC T cells differ from their adult counterparts. Specifically, in response to TCR activation, UC T cells exhibit impaired Ras activation, decreased expression of several activation markers, and diminished cytokine secretion (20–22).

UC, like bone marrow, contains a large number of hematopoietic stem cells allowing the hematopoietic system to be reconstituted in patients with cancers and genetic disorders. However, patients receiving UC transplants have a reduced level of severe graft-vs.-host disease as compared with individuals with bone marrow transplants (23). Graft-vs.-host disease, a major cause of mortality in patients with transplants, is caused by the activation of alloreactive T cells in the UC or bone marrow innoculum. Nevertheless, it remains to be determined whether the decreased severity of graft-vs.-host disease in individuals with UC blood transplants is caused by distinct characteristics of the naive lymphocytes present in UC-vs.-adult bone marrow or, alternatively, by the presence of memory T cells in the latter source.

Here, we show that UC and APB CD4$^+$ lymphocytes are distinct with regard to IL-7-induced cell cycle progression and proliferation. IL-7-treated naive UC lymphocytes undergo several divisions, whereas neither naive nor memory APB CD4$^+$ lymphocytes proliferate. By using these IL-7-treated lymphocyte populations, we have determined that cell cycle progression does not correlate with their susceptibility to infection with an HIV-1-derived vector. Previous work has shown that HIV-1 reverse transcription in CD4$^+$ lymphocytes depends absolutely on cell cycle entry (24), but it has been difficult to dissociate the requirements for cell cycle entry from the parameters governed by the state of T cell activation. Our data demonstrate that HIV-1-based vector infection in cytokine-stimulated lymphocytes is regulated by the global cellular environment.

Materials and Methods

Purification and Culture of Human CD4$^+$ T Cell Subsets. UC blood, obtained immediately after delivery of full-term infants, and APB, obtained from healthy adult donors, were collected in heparinized tubes. Peripheral blood mononuclear cells and UC mononuclear cells from fresh samples were separated over Ficoll–Hypaque (Sigma). Resting CD4$^+$ T cell subsets were purified by negative selection with α-glycophorin-A, α-CD8,

Abbreviations: UC, umbilical cord; APB, adult peripheral blood; γ_c, common gamma; 7AAD, 7-amino-actinomycin-D; CFSE, carboxyfluorescein diacetate succinimidyl ester; TCR, T cell receptor.

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α-CD14, α-CD20, α-HLADR, and α-CD69 in combination with either α-CD45RA or α-CD45RO monoclonal antibodies (Immunotech, Marseille, France) followed by addition of α-mouse IgG-conjugated magnetic beads (Dynal, Oslo). After a 30-min incubation at 4°C, samples were loaded on a magnetic column where antibody-bound cells were retained. Naive and memory populations were defined as the CD45RO and CD45RA subsets, respectively. The purity of each cell isolation was monitored on a FACScalibur (Becton Dickinson). Cells were then cultured in RPMI medium 1640 with 10% (vol/vol) FCS supplemented with IL-7 (10 ng/ml) (PeproTech, London). In some experiments, lymphocytes were stimulated with an immobilized α-CD3 antibody (UCHT1, 1 μg/ml; the generous gift of D. Cantrell, Imperial Cancer Research Fund, London) together with an α-CD28 antibody (9.3, 1 μg/ml; kindly provided by C. June, Univ. of Pennsylvania, Philadelphia).

Flow Cytometry for Surface Markers and Cell Cycle Analysis. To detect cell surface expression of IL-7Rα and γ_c chains, cells were incubated with the appropriate phycoerythrin-conjugated monoclonal antibody (Immunotech and PharMingen). Background fluorescence was measured by using an Ig isotype control antibody. Simultaneous detection of the cell surface CD45RO marker and enhanced green fluorescent protein (EGFP) was performed with an anti-CD45RO-ECD-conjugated monoclonal antibody (Immunotech). Cells were incubated with antibodies for 20 min on ice and then washed in PBS before fluorescence-activated cell sorter analysis.

Cell cycle analysis was performed by staining DNA and RNA with 7-amino-actinomycin-D (7AAD) and pyronin Y, respectively (24). A total of 5×10^5 cells were labeled sequentially with 7AAD (Calbiochem) at a final concentration of 20 μM for 30 min at room temperature and 5 μM pyronin Y (Polysciences) for 10 min on ice. Cells were immediately analyzed on a FACScalibur flow cytometer.

5-Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) Labeling. Freshly isolated $CD4^+$ T cells were washed and resuspended in PBS at a concentration of 2.5×10^6 cells per ml for labeling with the fluorochrome CFSE (Molecular Probes). Cells were incubated with CFSE at a final concentration of 2.5 μM for 3 min at room temperature. Labeling was terminated by the addition of FCS (30% of total volume), and cells were washed twice, then cultured as indicated.

T Cell Repertoire Analysis. Total RNA was prepared with the TRIzol reagent (GIBCO/BRL). RNA (2 μg) was reverse transcribed with random hexanucleotides (Amersham Pharmacia) with Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL). cDNAs were amplified (40 cycles) in a 25-μl reaction mixture with one of the 24 TCRBV subfamily-specific primers and a Cb primer recognizing the two constant regions Cb1 and Cb2 of the β chain of the TCR (25). Two microliters of the 24 TCRBV/Cb-first run PCR products were subjected to two cycles of elongation (run-off) with a Cb dye-labeled (6-Fam) primer allowing PCR products to be detected on an automated DNA sequencer (Applied Biosystems). Run-off PCR products were loaded on a 6% acrylamide sequencing gel and analyzed for size and fluorescence intensity with the IMMUNOSCOPE software. The TCRBV nomenclature proposed by Arden *et al.* was used in this study (26).

Virus Production and Retroviral Infections. All infections were performed by using an HIV-1-based vector that was modified to include a 178-bp fragment encompassing the central polypurine tract and the central termination sequence, the EGFP reporter gene downstream of the EF1α promoter, and a 400-bp deletion in the U3 enhancer (TRIPΔU3-EF1α) (27). Virions were produced by transient calcium phosphate cotransfection of 293T cells with the vector plasmid, an encapsidation plasmid lacking accessory HIV-1 proteins (p8.91), and a vesicular stomatitis virus G protein (VSV-G) envelope expression plasmid (pH-CMV-G) (28, 29). The concentration of virion particles was normalized by measuring the P24 (HIV-1 capsid protein) content of supernatants by ELISA. Lymphocytes (1×10^6) were incubated with lentiviral vector particles, at a concentration corresponding to 50 ng of viral p24/ml, for 12 h in RPMI medium 1640 containing recombinant IL-7 on fibronectin-coated plates (27). Cells were then centrifuged and resuspended in fresh medium with IL-7. Under conditions where cells were stimulated through the TCR, infections were performed in the presence of recombinant IL-2. Transduction efficiencies were assessed 48 h later. Infected cells were identified by their EGFP fluorescence on a FACScalibur.

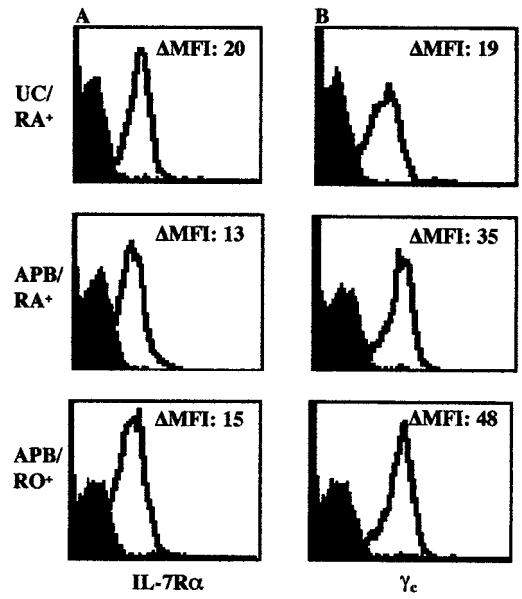

Fig. 1. Expression of the IL-7R differs on UC and APB $CD4^+$ T cells. Naive (RA^+) and memory (RO^+) $CD4^+$ resting T lymphocytes were isolated from UC and APB by negative selection. Expression of the IL-7Rα (*A*) and γ_c chains (*B*) on the purified $CD4^+$ T cell subsets was analyzed on a FACScalibur with phycoerythrin-conjugated monoclonal antibodies (open histograms). The Ig isotype control (filled histograms) and the change in mean fluorescence intensity (ΔMFI) are indicated in each histogram. Data are representative of results obtained in four independent experiments.

Results

IL-7R Expression in $CD4^+$ T Cell Subsets. To assess the role of IL-7 in UC and APB $CD4^+$ T cells, it was first necessary to monitor receptor levels on these lymphocyte subsets. The IL-7R is composed of the IL-7Rα chain as well as the γ_c chain. Although the IL-7Rα chain was expressed on all lymphocyte subsets, levels were ≈1.5-fold higher on naive UC $CD4^+$ T cells than on either memory or naive APB $CD4^+$ T cells ($P < 0.05$). However, in agreement with previous reports (30), expression of γ_c was significantly higher on both APB $CD4^+$ T cell subsets than on UC lymphocytes (Fig. 1) ($P < 0.001$). These data suggest that the responses of UC and APB to cytokines that signal through γ_c may differ. Nonetheless, because there was no correlation between the surface levels of the two IL-7R subunits in UC vs. APB populations, it was not possible to predict the responsiveness of a given subset to IL-7 stimulation.

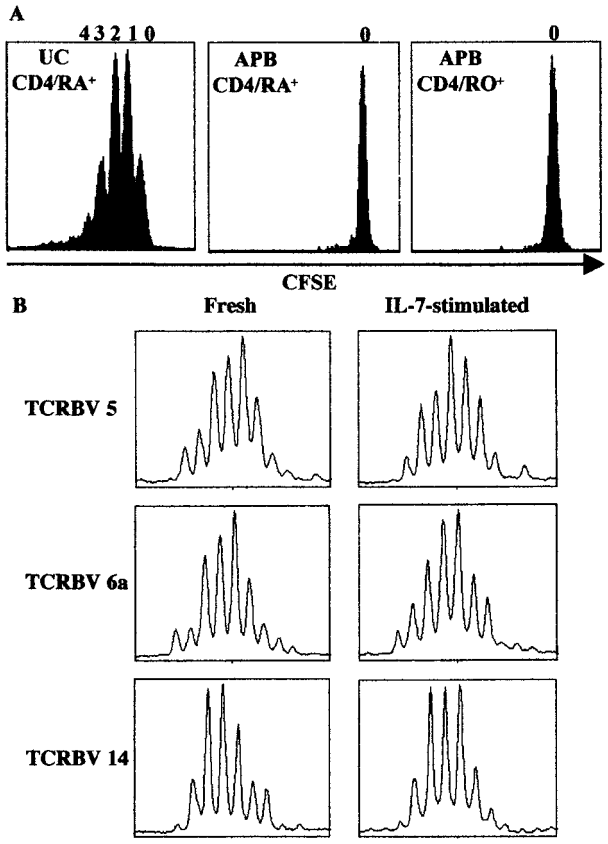

Fig. 2. IL-7 drives the proliferation of naive UC but not APB CD4$^+$ T cells. (*A*) Naive CD4$^+$ UC T lymphocytes (CD4/RA$^+$) and naive as well as memory CD4$^+$ APB T lymphocytes (CD4/RA$^+$ and CD4/RO$^+$, respectively) were purified, labeled with CFSE, and cultured *in vitro* in the presence of IL-7 (10 ng/ml). After 6 days, cells were analyzed for CFSE intensity by flow cytometry. The numbers shown above the peaks indicate the number of cell divisions. Data are representative of results obtained with CD4$^+$ T cells isolated from eight UC and APB donors. (*B*) TCR complementary determining region 3 (CDR3) size distribution (Immunoscope profiles) of freshly isolated and IL-7-stimulated CD4$^+$ UC T cells (9 days). Products were generated by reverse transcription PCR with 24 different TCRBV and 1 constant β consensus primer (Cb), followed by a run-off reaction with a fluorescent Cb primer. Examples of three representative TCRBV subfamilies from freshly isolated and IL-7-stimulated CD4$^+$ UC lymphocytes are shown.

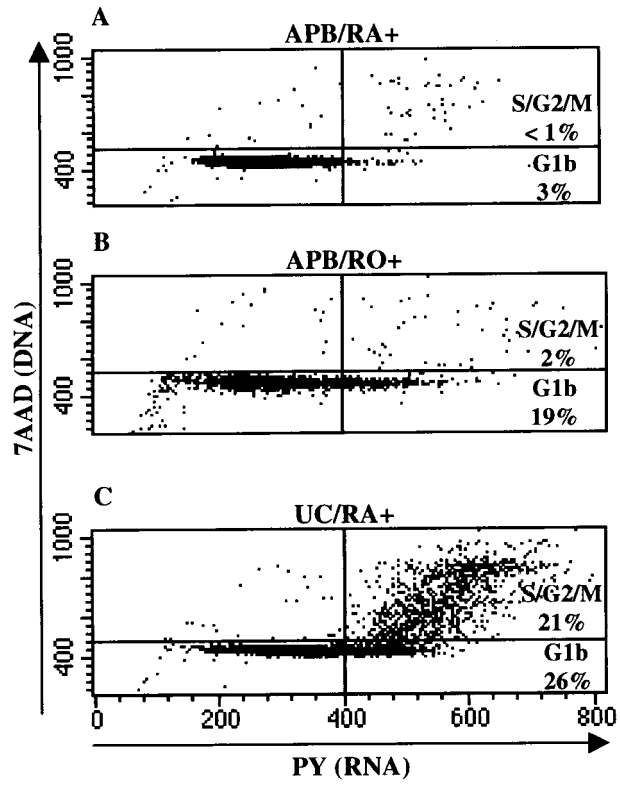

Fig. 3. Comparison of cell cycle entry in naive CD4$^+$ APB, memory CD4$^+$ APB, and naive CD4$^+$ UC lymphocytes after IL-7 stimulation. Naive CD4$^+$ APB (*A*), memory CD4$^+$ APB (*B*), and naive CD4$^+$ UC (*C*) cell populations were analyzed for cell cycle progression after stimulation with IL-7. DNA and RNA levels were measured by 7AAD and pyronin Y (PY) staining, respectively. In each dot blot, cells to the left of the inserted vertical line have not yet entered the G_{1b} phase of the cell cycle. The percentages of cells in the G_{1b} and $S/G_2/M$ stages are indicated. Data are representative of IL-7-induced cell cycle progression after 6–10 days of *in vitro* culture and were obtained in four independent experiments.

Proliferation and Cell Cycle Progression in IL-7-Stimulated CD4$^+$ T Cell Subsets. Purified naive and memory UC and APB CD4$^+$ T cells were labeled with CFSE to study IL-7-induced proliferation and generation of daughter cells. Naive UC CD4$^+$ T cells proliferated after IL-7 treatment, undergoing about four divisions by day 6 after stimulation (Fig. 2*A*). Similar levels of proliferation were observed in naive UC T cells isolated from eight different donors. To determine whether IL-7 induces the proliferation of a polyclonal population of UC CD4$^+$ T cells, we compared the TCR repertoire of freshly isolated UC CD4$^+$ lymphocytes with the corresponding IL-7-stimulated population by using the Immunoscope method (25). After IL-7 stimulation, there was no skewing of distinct TCR β sequences; all TCRBV families were present and displayed a Gaussian-like profile. Thus, a polyclonal population of UC CD4$^+$ T cells proliferated in response to IL-7 (Fig. 2*B*).

In marked contrast to the proliferation observed in UC CD4$^+$ cells, neither naive nor memory CD4$^+$ APB lymphocytes divided after IL-7 treatment, as assessed in eight different donors (Fig. 2*A* and data not shown). Nevertheless, these cells responded to IL-7, because in agreement with previous reports, their survival was promoted by this cytokine (15–17). To ensure that the lack of an IL-7-induced proliferative responses in APB T cells was not simply caused by a decreased ability to respond to IL-7, internalization of the IL-7R was monitored. The level of the IL-7Rα subunit decreased to almost undetectable levels in APB and UC CD4$^+$ T cells after a 24-h culture in the presence of IL-7 (data not shown). The concentration of IL-7 in the culture media (10 ng/ml) was therefore sufficient to saturate the IL-7R in all three T cell populations. Thus, APB CD4$^+$ lymphocytes respond to IL-7 under the *in vitro* conditions used here.

To determine whether IL-7 modulated cell cycle progression in APB T cell subsets, a technique that permits simultaneous DNA and RNA quantitation by flow cytometry (staining with 7AAD and pyronin Y, respectively) was used (24). Almost all freshly isolated T lymphocytes are in the G_0 resting state, but on activation with IL-7, some of these lymphocytes progressed to G_{1b} (Fig. 3*B*). G_{1b} is defined as the stage at which RNA levels increase before entering S phase. Cell cycle transit of APB lymphocyte populations was not equivalent. Significantly higher numbers of memory CD4$^+$ cells progressed through G_{1b} (Fig. 3). Even in the memory APB CD4$^+$ population, transition into $S/G_2/M$ occurred either very slowly or not at all. As expected from the data obtained in the CFSE proliferation experiments, naive UC CD4$^+$ T cells entered G_{1b} and then progressed into $S/G_2/M$ (Fig. 3*C*).

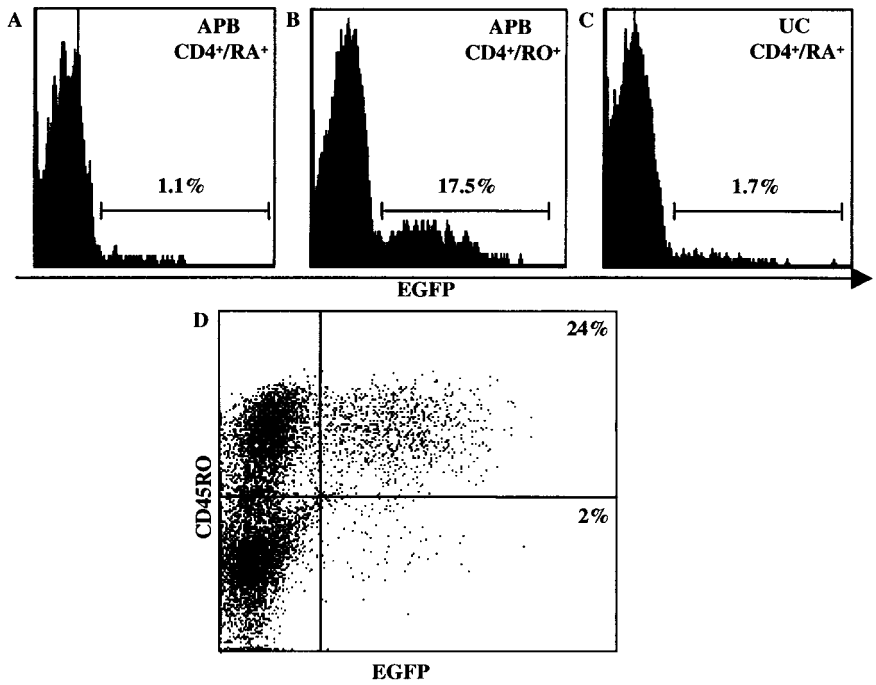

Fig. 4. Memory $CD4^+$ APB lymphocytes are significantly more susceptible to infection with an HIV-1-based vector. Naive APB (*A*), memory APB (*B*), and naive UC $CD4^+$ T cells (*C*) were stimulated in culture with IL-7 for 4 days. Cells were then infected with VSV-G-pseudotyped HIV-1-derived virions expressing the EGFP transgene (corresponding to 50 ng p24/ml) for a 12-h period. Cells were analyzed by flow cytometry 2 days after infection, and the percentage of $EGFP^+$ cells is indicated in each histogram. Data are representative of results obtained in six separate experiments. (*D*) To compare the infection of naive and memory T cells directly, nonsorted APB T cells were stimulated with IL-7 and infected as described above. After 2 days, infection in the memory and naive subsets was assessed by simultaneously monitoring EGFP fluorescence and CD45RO expression with an ECD-conjugated antibody. The percentage of $EGFP^+$ infected memory ($CD45RO^+$) and naive ($CD45RO^-$) T lymphocytes is indicated in the upper right and the lower right quadrant, respectively.

Infection of IL-7-Stimulated $CD4^+$ Lymphocytes with an HIV-1-Based Vector. The productive infection of both naive and memory $CD4^+$ T cells with HIV-1 requires a transition into the G_{1b} stage of the cell cycle (24). As indicated above, most circulating peripheral T lymphocytes are in the G_0 resting state; thus, a condition that would theoretically preclude HIV-1 infection. It is therefore assumed that, *in vivo*, HIV-1 infection is limited to $CD4^+$ T cells that are cycling, because they have recently been stimulated by their cognate antigen. One quandary that arises from this hypothesis is that those T cells stimulated through their TCR are, by definition, activated lymphocytes. The presence of HIV-1 in "nonactivated" naive T cells *in vivo* (31–33) raises important issues regarding the mechanisms controlling HIV-1 infection in this population. One possibility is that productive HIV-1 infection can occur after exposure of $CD4^+$ lymphocytes to IL-7. IL-7 does not alter expression of surface markers (refs. 15, 17, 19 and 34; data not shown). Thus, naive lymphocytes retain their "naive" phenotype, and activation markers are not induced on the memory population.

Because IL-7-induced entry into the G_{1b} phase of the cell cycle differed in each of the three $CD4^+$ lymphocyte populations studied here, it was possible to assess whether cell cycle progression was sufficient for HIV-1 infection. To evade the confounding variables arising from differences in HIV-1 coreceptor levels on naive and memory T cells (35, 36), an HIV-1-vector pseudotyped with VSV-G was used. The VSV-G receptor is expressed at high levels on all cell types and does not appear to be altered with the activation state of a cell (28). The EGFP cDNA was subcloned downstream of the ubiquitous cellular EF1α promoter such that infection could be easily monitored and expression of the EGFP reporter would not be modulated by the activation state of the cell (27). Finally, the HIV-1-vector harbors the central polypurine tract and the central termination sequence of HIV-1, sequences which act as a cis-determinant of HIV-1 genome nuclear import (37).

Memory APB $CD4^+$ lymphocytes were infected with the HIV-1-vector at significantly higher levels than the naive APB subset (Fig. 4 *A* and *B*). The important variability in infection was also reproduced when lymphocytes were not purified on the basis of memory and naive markers, with 10-fold higher levels of infection in the memory $CD45RO^+$ population than in the naive $CD45RO^-(RA^+)$ population (Fig. 4*D*). Thus, differences in the susceptibility of these subsets to HIV-1-vector transduction were not caused by an artifact related to the purification procedure. Collectively, our data indicate that IL-7 can generate conditions conducive for HIV-1-vector infection in resting $CD4^+$ memory, but not naive, APB lymphocytes. The level of HIV-1-vector infection in naive UC $CD4^+$ lymphocytes was as low as that observed in the naive APB counterpart (Fig. 4*C*). Because UC lymphocytes underwent significant proliferation in response to IL-7, these results demonstrate that IL-7-induced G_{1b} progression is not sufficient for lentiviral-based vector infection in this population.

Proliferation and HIV-1-Based Vector Infection of TCR-Stimulated $CD4^+$ Lymphocytes. To determine whether the resistance of naive UC lymphocytes to HIV-1-vector infection was a peculiarity of IL-7 stimulation, these cells were also infected after TCR engagement. Infection of CD3/CD28-stimulated $CD4^+$ UC lymphocytes ranged from 40 to 60%, levels equivalent to those observed in $CD4^+$ APB lymphocytes (Fig. 5*A*). This substantial infection occurred in the context of a high level of proliferation in all $CD4^+$ populations, with the emergence of three generations of daughter cells after 3 days of stimulation (Fig. 5*B*). Thus,

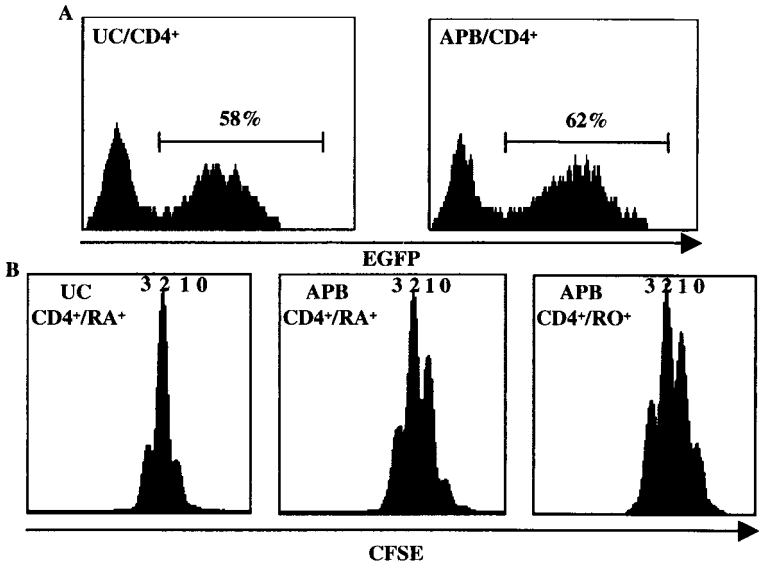

Fig. 5. HIV-1-based vector infection and proliferation in UC and APB CD4$^+$ T cells after TCR activation. (*A*) CD4$^+$ UC and APB lymphocytes were stimulated with immobilized anti-CD3 and anti-CD28 antibodies for 2 days and then infected with VSV-G-pseudotyped HIV-1 virions during a 12-h period. EGFP expression was analyzed 48 h later. (*B*) CFSE-labeled naive UC, naive APB, and memory APB CD4$^+$ T cells were stimulated for 3 days as indicated, and the number of generations of dividing cells was assessed by flow cytometry. Data are representative of results obtained in four independent experiments.

the susceptibility of naive UC CD4$^+$ lymphocytes to HIV-1-vector infection is a function of the activation state of the cell. In this regard, it is important to note that the activation profile of IL-7- and TCR-stimulated T cells differed significantly: IL-7 stimulation did not result in expression of the CD69 surface marker in any of the three CD4$^+$ T cell subsets, and the IL-2Rα subunit was expressed at only minimal levels, whereas both these proteins were expressed at high levels in TCR-engaged CD4$^+$ lymphocytes (data not shown).

Discussion

Several recent reports have sought to elucidate the role of the IL-7 cytokine in human postthymic development (15–17). It has been determined that *in vitro*, IL-7 functions as a survival factor for both naive and memory T cells without modifying their differentiation and maturation state. Thus, IL-7 is likely to contribute to the size of the recirculating T cell pool and, more specifically, to the presence of naive lymphocytes in the periphery. Our data shed new light on the role of IL-7 in promoting the homeostatic expansion of naive CD4$^+$ lymphocytes. The effects of IL-7 on naive UC and APB CD4$^+$ cells are strikingly different; only naive UC CD4$^+$ lymphocytes proliferated in response to IL-7. One distinction between UC lymphocytes and their APB counterpart is that UC lymphocytes are mainly recent thymic emigrants. Our results suggest that the presence of naive T cells in the peripheral circulation for long periods may be promoted by an initial IL-7-induced proliferation of recent thymic emigrants. These data are supported by results from the murine model demonstrating that recent thymic emigrants have a selective survival advantage over resident peripheral naive T cells for up to 4 weeks after thymic export (38). Thymic function is not restricted to the neonatal period; a substantial output of T cells continues until late adulthood (39). Collectively, our observations have important implications for patients undergoing transplantation with UC, bone marrow, or mobilized peripheral blood as a source of stem cells. Many of these patients, who are lymphopenic immediately after transplantation, have significantly elevated serum IL-7 levels (40). It is therefore expected that, under these conditions, IL-7 plays a role in enhancing the homeostatic expansion of the newly differentiated T cells.

The restricted lymphokine profile of naive T lymphocytes after TCR stimulation has been invoked to explain the decreased susceptibility of this T cell subset to productive HIV-1 infection (31, 41, 42). Nevertheless, the different levels of HIV-1 infection in naive and memory T cell subsets may also be due to important variations in the level of HIV-1 coreceptors on the surface of these cells (35, 36). With use of an HIV-1-derived vector pseudotyped with a ligand whose receptor is ubiquitous on all cell types (VSV-G), it was possible to discern whether integration and transgene expression from a lentiviral vector were regulated at the level of cell cycle entry or, alternatively, by the activation state of the cell. Because IL-7-treated CD4$^+$ UC lymphocytes were significantly less susceptible to infection with a VSV-G-pseudotyped HIV-1 vector than were memory APB lymphocytes, IL-7-induced cell cycle progression and proliferation are not an absolute gauge of the susceptibility of a cell to HIV-1-vector infection. However, we and others have shown that infection of T lymphocytes with an HIV-1-based vector cannot occur in the absence of cell cycle progression (18, 27).

The relative resistance of proliferating IL-7-treated UC CD4$^+$ lymphocytes to lentiviral infection is supported by recent work showing that cell cycle progression is required but not sufficient for HIV-1 infection after TCR activation (24, 43). Host factors such as NFATc and c-Myc appear to regulate HIV-1 reverse transcription and nuclear import via a mechanism distinct from that controlling cell cycle entry (43, 44). In our system, although both IL-7R- and TCR-mediated signals induced proliferation of UC CD4$^+$ lymphocytes, only activation via the latter cascade generated a cellular environment conducive to lentiviral vector infection. In contrast, memory APB lymphocytes, which proliferated only minimally to IL-7, have apparently matured in such a manner that the IL-7 signal created permissive conditions for infection. These data have important implications for gene therapy trials with HIV-1-based vectors and may further our understanding of wild-type HIV-1 infection in CD4$^+$ lymphocyte subsets that are not stimulated through their cognate antigen.

The data presented here differ from those of Unutmaz and colleagues (18), who recently reported equivalent levels of HIV-1-vector infection in naive and memory CD4$^+$ lymphocytes, but this discrepancy is likely due to significant technical differences. Specifically, these researchers infected cells with VSV-G-pseudotyped virions for 5 consecutive days, whereas the cells used here were infected only once for a 12-h period. Multiple rounds of infection may have been obligatory, because previous generations of HIV-1-derived vectors were not optimized for nuclear import in T lymphocytes (27, 37). In this regard, it is also intriguing to note that the manipulation and

handling of human blood samples can significantly bias experimental outcome. Specifically, the type of anticoagulant and the timing between blood draws and lymphocyte isolation can modify the level of expression of cytokine receptor subunits, including those constituting the IL-7R (45).

Our results support a view of naive UC and APB lymphocytes as functionally disparate. UC lymphocytes differ from their naive APB counterpart in that the former represent, almost solely, recent thymic emigrants. The high proliferative potential of UC lymphocytes in response to IL-7 suggests that this cytokine contributes to the maintenance of a diverse T cell repertoire in the periphery.

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