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J Immunol 2003; 171:5208-5214; doi: 10.4049/jimmunol.171.10.5208
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Impaired TCR-Mediated Induction of Ki67 by Naive CD4\(^+\) T Cells Is Only Occasionally Corrected by Exogenous IL-2 in HIV-1 Infection\(^1\)

Scott F. Sieg,\(^2\) Douglas A. Bazdar, and Michael M. Lederman

Perturbations in naive T cell homeostasis and function may play a major role in the immunodeficiency that accompanies HIV infection. By examining naive CD4\(^+\) T cell function on a single cell basis, we provide evidence that these cells have significant qualitative defects in HIV disease. Ki67, a molecule expressed during cell cycle progression, is induced less efficiently among naive CD4\(^+\) T cells from HIV-infected individuals following activation with anti-TCR Ab. The impairment in Ki67 expression is evident even when a separate function, CD62L down-modulation, is within normal ranges. Moreover, the defects in Ki67 induction are only sometimes corrected by the addition of rIL-2 to cell cultures. An initial assessment of IL-2 unresponsiveness in cells from selected HIV-infected individuals suggests that the defect is not a consequence of impaired IL-2R expression or IL-2R signaling capability. Qualitative defects in naive T cells that cannot be routinely corrected by IL-2 have significant implications for disease pathogenesis and for strategies using IL-2 as a vaccine adjuvant in HIV disease. *The Journal of Immunology*, 2003, 171: 5208–5214.

Naive T cells express high levels of the CD45RA isofrom and high levels of CD62L, which distinguish these cells from the various subsets of effector and central memory T cells (1, 2). Analyses of cells expressing these markers clearly demonstrate that naive CD4 and CD8\(^+\) T cells are progressively depleted during the course of HIV infection (3–5). The loss of naive T cells could severely limit the capacity of patient immune systems to respond to novel Ags including those that may result from escape mutations by replicating HIV. Depletion of naive T cells appears to predict clinical outcome (6), responsiveness to therapy (7), and may also predict poor immunization responses by HIV-infected patients (8). Thus, naive T cell depletion in HIV disease has significant clinical implications.

Although studies of naive T cell depletion in HIV disease have been informative, less is known regarding the qualitative changes in naive T cells that may occur during HIV infection. One possible indicator of naive T cell function is proliferation response to mitogenic stimulation. These responses appear to be reduced in whole PBMC (9–11) and in CD45RO-depleted PBMC cultures (12) from HIV-infected individuals. However, some investigators, who have demonstrated naive T cell depletion in HIV disease using CD45RA and CD62L expression as markers of naive T cells, have argued that these apparent proliferation defects in response to mitogen stimulation may simply be a consequence of reduced naive T cell precursor frequencies in PBMC from HIV-infected individuals (4). Thus, naive T cell proliferation function in HIV disease remains largely undefined.

Ag-induced naive T cell proliferation depends on signals from TCR activation that lead to several functional and phenotypic changes including induction of the T cell growth factor, IL-2. This cytokine then binds to the IL-2R complex to facilitate cell cycle progression. The IL-2R complex consists of inducible CD25 (\(\alpha\)-chain) and constitutively expressed CD122 (\(\beta\)-chain), and CD132 (\(\gamma\)-chain). The latter molecules mediate receptor signaling via Janus kinase (Jak)\(^3\)/STAT activation (13–17). Janus kinases (Jak1 and Jak3) are protein tyrosine kinases that associate with CD122 and CD132 and upon IL-2R ligation, these kinases mediate phosphorylation of the IL-2R. The phosphorylated IL-2R chain (CD122) then serves as a docking site for STAT5 A and B proteins, which are subsequently activated by Jak. Both Jak1 and Jak3 appear to be required for normal activation of STAT5 (18, 19). Once activated, STAT proteins dimerize and translocate to the nucleus where they bind DNA and mediate gene transcription. The possibility that defects in IL-2R expression or signaling may be involved in the proliferation failure of T lymphocytes that occurs with HIV infection needs to be considered.

It is generally appreciated that patient T cells have less potential to produce IL-2 in response to TCR activation (9, 20), but the relative responsiveness of patient T cells to IL-2 stimulation is less clear. The relationship between IL-2R expression and proliferation function has been partially characterized in HIV patients such that induced CD25 expression is not necessarily a predictor of subsequent T cell proliferation (21). Analyses of CD122 and CD132 expression in HIV disease have been controversial (22–24), probably stemming from difficulties in assessing surface expression of these molecules by standard flow cytometry techniques. Thus, the relationships among expression of IL-2R, signaling function, and proliferation defects in patient naive T cells are unknown.

To address these issues, we have utilized anti-V\(\beta\)3 Ab stimulation to evaluate naive T cell proliferation function on a single cell basis. This agonistic Ab is specific for the variable \(\beta\)3 (V\(\beta\)3) chain.
of the TCR and activates 3–5% of peripheral blood T cells without
the bystander effects that occur as a result of stimulation with more
broadly mitogenic agents (21, 25). T cells stimulated by this Ab
can be readily identified by flow cytometry and further evaluated
for phenotypic and functional characteristics. Comparison between
anti-Vβ3 and anti-CD3 Ab stimulation demonstrates that anti-Vβ3
Ab stimulation provides a more sensitive probe for detecting prolif-
eration defects in PBMs from HIV-infected patients and that the
proliferation defect is characterized by a G1 phase cell cycle arrest
despite induction of the early activation markers, CD69 and CD25
with magnetic microbeads (Miltenyi Biotec, Bergish Gladbach, Germany).

Cells from HIV-infected individuals demonstrate that naive CD4
phases of the cell cycle and remains elevated throughout mitosis (26). Although the func-
tion of this molecule is not entirely defined, Ki67 molecules are
physically associated with nucleoli within the cell and antisense experiments indicate that Ki67 expression may be important for
cellular proliferation and survival. Importantly, Ki67 is not ex-
pressed in resting cells (27), thereby making this molecule a rea-
sonable marker of cell cycle progression.

Our analyses of induced Ki67 expression among naive CD4+ T
cells from HIV-infected individuals demonstrate that naive CD4+
T cells from HIV-infected patients have qualitative defects in cell
cycle progression that cannot always be rescued by the addition of
rIL-2 to cell cultures. This qualitative defect in naive CD4+ T cells is not related to impairments in expression of IL-2R or to impair-
ments in IL-2-induced signaling. Naive CD4+ T cell defects may
contribute to the progressive immune deficiency in HIV disease
and also may limit the effectiveness of vaccination strategies in
HIV infection.

Materials and Methods

Cells

PBMC were acquired from HIV-infected individuals and healthy donors.
Patients having fewer than 50 CD4 cells/μl were excluded because not
enough cells could be recovered for analysis. No other exclusion criteria
were used. PBMC were isolated from whole blood by centrifugation over
a Ficoll-Histopaque cushion and depleted of CD45RO or CD8+ T cells
with magnetic microbeads (Miltenyi Biotec; Bergish Gladbach, Germany).
For depletions, cells were incubated for 15 min with anti-CD45RO and
anti-CD8 microbeads at 4°C, washed in PBS, and passed through magnetic
bead separation columns. Cells were cultured in RPMI 1640 (BioWhit-
taker, Walkersville, MD) supplemented with 10% FBS, 2 mM L-glutamine,
and antibiotics.

Ki67 expression

CD45RO-CD8-depleted PBMC were stimulated with anti-Vβ3 Ab (BD
PharMingen, San Diego, CA; 100 ng/ml) in the presence or absence of
rIL-2 (Chiron, Emeryville, CA; 360 U/ml). The dose of rIL-2 used for these
studies was based on preliminary experiments that determined the concen-
tration of rIL-2 needed to consistently restore proliferation function in anti-
Vβ3 Ab-stimulated cells from healthy donors that were treated with the
immunosuppressive drug, cyclosporin A (data not shown). After 2 days of
culture, cells were surface stained with fluorescein-labeled Abs (BD
PharMingen) to CD4, Vβ3, and CD26L by incubating the cells at room
temperature for 10 min with Abs in PBS/BSA/azide solution (staining buff-
er). Intracellular staining for Ki67 was conducted by incubating surface
stained cells with FACS permeabilizing solution (BD Biosciences, San
Jose, CA) and then exposing cells to anti-Ki67 Ab (BD PharMingen). After
incubating, cells were washed with staining buffer and analyzed on a flow
cytometer (FACSCalibur; BD Biosciences).

Enzyme amplification flow cytometry

CD45RO-CD8-depleted PBMC that had been incubated without stimula-
tion overnight were analyzed the next day for CD122 and CD132 expres-
sion by enzyme amplification flow cytometry. Cells were surface stained
for CD4, CD62L, and Vβ3 by standard techniques and for either CD122 or
CD132 by enzyme amplification flow cytometry as outlined in the com-
mercially available kit (Flow-Amp Systems, Cleveland, OH). Biotin-con-
jugated anti-CD122 and anti-CD132 or isotype control Abs were incubated
with the cells. Cells were washed, labeled with streptavidin-HRP for 10
min at room temperature, washed again and then incubated in amplification
substrate that deposits biotin-conjugated tyramide radicals (produced
by enzymatic reaction with HRP-conjugate) on the cell surface. Next, cells
were exposed to Streptavidin-FITC and analyzed on the flow cytometer. Isotype-control Abs were used to judge background staining for CD122
and CD132 expression.

STAT5 phosphorylation

STAT5 phosphorylation was examined by flow cytometry using a previ-
ously described method (28). Briefly, CD45RO-CD8-depleted PBMC that
had been unstimulated and cultured overnight in medium alone were
exposed to rIL-2 (Chiron, 360 U/ml) or left unstimulated for 15 min. Cells
were then treated with formaldehyde solution, washed, fixed in 90% meth-
anol and then stained with anti-Vβ3 (IgG2a isotype) and anti-P-STAT5 Ab
(IgG1 isotype). After a washing in staining buffer, the cells were incubated
with a FITC-conjugated, goat anti-mouse IgG1 Ab that did not cross-react
with IgG2a Ab. Cells were washed again and examined by flow cytometry.

Results

Naive CD4+ T cells from HIV-infected individuals have
impaired Ki67 expression after TCR stimulation that is variably
corrected by recombinant IL-2

To avoid confounding issues associated with positive selection of
cells for subset studies for functional studies, we used negative selection
and examined changes in the naive T cell phenotypic marker
CD62L to assess naive T cell activation in vitro. PBMC were
depleted of CD8+ and CD45RO+ cells and the remaining cells were
stimulated with anti-Vβ3 Ab with or without rIL-2. Depletions
resulted in cell populations having predominantly a
CD45RAhigh phenotype with a small contribution (generally in the
range from 5 to 15%) from CD45RA+ cells. Essentially all
CD45RA+/CD45RO+ cells were depleted. After 2 days, the cells
were examined for Ki67 expression as an indicator of cell cycle
progression and for CD62L to evaluate naive T cell phenotype and
activation. Preliminary experiments using anti-Vβ3 Ab to stimu-
late PBMC from two healthy donors and four HIV-positive pa-
tients indicated that 2–4 days after stimulation, both induced Ki67
expression and incorporation of bromo-deoxyuridine (an indica-
tion of cells in S phase) occurred almost exclusively among the
Vβ3+ cells and that the bromo-deoxyuridine+ cells were consis-
tently Ki67+ (data not shown). Thus, induced Ki67 expression is
tightly linked to cell cycle progression in this model.

Following stimulation of isolated cells, naive CD4+ Vβ3+ T
cells (defined as CD62Lhigh cells in CD45RO-depleted cultures)
tend to lose expression of CD62L and gain expression of Ki67
(Fig. 1). In contrast to cells from healthy donors, cells from HIV-
infected individuals demonstrated heterogeneous responses, with
cells from some patients demonstrating a relative failure to express
Ki67, whereas cells from other patients had normal responses. The
addition of rIL-2 could not always restore the responses of cells
from HIV-infected individuals to levels observed in cells from
healthy donors (Figs. 1 and 2).

To evaluate more specifically the induction of Ki67 expression
by phenotypically defined naive T cells, HIV-infected subjects
were categorized into two groups based on the percentages ofphe-
notypically naive (CD62Lhigh) CD4+Vβ3+ cells in CD8-
CD45RO-depleted PBMC cultures. The proportion of CD4+Vβ3+
cells with the CD62Lhigh phenotype in samples prepared from
healthy controls (n = 16) ranged from 85 to 99.4% (>85%).
Therefore, we divided patients into those whose CD62Lhigh-
expressing CD4+Vβ3+ cells fell within this range (>85%; range,
85 to 96%) and those who had abnormally low levels of CD62Lhigh-
expressing cells after purification by negative selection (<85%;
range, 38 to 81%). Patients with reduced (<85%) proportions of
CD62Lhigh CD4+Vβ3+ cells after CD45RO-depletion tended to
have lower CD4 cell counts (mean, 336 vs 472, p < 0.01) than the patients with normal representation of naive T cells. These patients also had reduced plasma HIV RNA levels (mean, 28,000 vs 102,146 copies per ml, p < 0.01). The latter was probably due to the fact that a higher proportion of these individuals were receiving antiretroviral therapy (70 vs 30%) at the time of the study. Patient age (mean, 40–42 years) and infrequent representation of females (~5% for each group) were not different between the patient populations.

Analysis of Ki67 induction following stimulation with anti-Vβ3 Ab was determined by subtracting the percentages of CD4+ Vβ3+ that expressed Ki67 in cultures that had not been stimulated from the percentages of CD4+ Vβ3+ cells that expressed Ki67 after stimulation with anti-Vβ3 Ab (Δ Ki67). Induced Ki67 expression was impaired in cells from both groups of patients as compared with Ki67 induction in cells of the control group following TCR stimulation with or without rIL-2 (Fig. 2). Although there was a tendency for poorer responses in the presence of IL-2 from the patient population with fewer naive T cells, this was not statistically different from the response of patients who had "normal" naive T cell precursor frequencies. Thus, neither failure to express Ki67 after TCR stimulation nor the suboptimal restoration of function by rIL-2 could be explained by reduced naive T cell precursor frequencies in these CD45RO-depleted cells.

We next examined the relationship between TCR-induced Ki67 expression and TCR-induced CD62L down-modulation among the CD4+ Vβ3+ cells. Analyses of cells from patients with normal levels of naive CD4+ Vβ3+ cells after CD45RO-depletion revealed that these cells had minor, statistically insignificant reductions in the ability to down-modulate CD62L following TCR stimulation (mean responses, 36.8 vs 42.0% of CD4+ Vβ3+ cells from patients and controls, respectively, decreased CD62L expression with stimulation; p = 0.23, Student’s two-tailed t test). Nonetheless, selection of samples from only those patients whose cells decreased CD62L expression normally following TCR stimulation demonstrated a defect in induction of Ki67 expression that was not reliably corrected by rIL-2 (Fig. 3). Thus, the impaired induction of Ki67 expression represents a selective defect observed in naive T cells from some HIV-infected individuals.

**IL-2 unresponsiveness is not a consequence of decreased IL-2R expression or reduced signaling capacity by IL-2Rs**

Because the addition of rIL-2 failed to restore induction of Ki67 expression among activated naive CD4+ T cells from certain patients, we considered the possibility that there may be defects in IL-2 responsiveness in cells from these HIV-infected individuals. The signaling components of the IL-2R include CD122 and CD132. To clarify the role of CD122 and CD132 IL-2R expression and signaling in patients whose cells failed to respond to TCR stimulation even when rIL-2 was provided, we examined a subset of six HIV-infected patients and three healthy controls for their responses to TCR stimulation with or without rIL-2 with the intent of comparing these responses to IL-2R expression and function. Induction of Ki67 expression by TCR ligation was relatively poor 2 days poststimulation as compared with cells incubated in medium alone. Mean responses (Δ Ki67) of healthy donors were significantly different from mean responses from both patient groups in the presence or absence of IL-2 (p = <0.01 by Student’s two-tailed t test).

**FIGURE 1.** Induction of Ki67 and down-modulation of CD62L upon TCR stimulation. PBMC were depleted of CD45RO and CD8+ cells by use of magnetic beads and then were incubated with anti-Vβ3 Ab ± rIL-2 (360 U/ml). Histograms represent the levels of CD62L expression (y-axis) and Ki67 expression (x-axis) on CD4+ Vβ3+ cells after 2 days of culture with or without stimulation.

**FIGURE 2.** Reduced induction of Ki67 expression among patient cells and incomplete recovery with rIL-2. CD45RO-CD8-depleted PBMC were incubated with or without anti-Vβ3 Ab ± rIL-2 for 2 days. CD4+ Vβ3+ cells were assessed for the expression of CD62L and Ki67 by flow cytometry. Patients were grouped according to the levels of CD4+ Vβ3+ cells that were CD62Lhigh cells (naive) in unstimulated cultures. Cells from patients who had relatively normal frequencies of CD62Lhigh cells (>85%) and cells from HIV+ individuals with lower levels (<85%) were compared with cells from healthy controls (CD62Lhigh >85%). The Δ Ki67 represents the relative increase in the percentages of CD4+ Vβ3+ cells that expressed Ki67 2 days poststimulation as compared with cells incubated in medium alone. Mean responses (Δ Ki67) of healthy donors were significantly different from mean responses from both patient groups in the presence or absence of IL-2 (p = <0.01 by Student’s two-tailed t test).
controls in response to rIL-2 and the levels of STAT5 phosphorylation did not distinguish between patients whose cells had relatively normal or poor Ki67 expression in response to stimulation with anti-Vβ3 with or without rIL-2 (Fig. 6, A and B). These results indicate that patient naive CD4+ T cells maintain the machinery required to mediate the early signaling events associated with IL-2 stimulation but may still fail to appropriately move through cell cycle (as indicated by the expression of Ki67) when activated by TCR stimulation in the presence of IL-2.

**TCR-induced CD25 expression and CD62L down-modulation are related to IL-2 responsiveness**

CD25 is an inducible IL-2R subunit that does not have intrinsic signaling capability. CD25 expression, although not essential for rIL-2-induced phosphorylation of STAT5, may still contribute to differences in IL-2 responsiveness by increasing the affinity of the IL-2R complex for its ligand (17) or by localizing high affinity receptors to microdomains within the plasma membrane (31). Therefore, we examined the relationship between CD25 expression and induced Ki67 expression. CD45RO-depleted cells were stimulated with anti-Vβ3 Ab and incubated for 48 h. The CD4+Vβ3+ cells were then analyzed for CD62L and CD25 expression or for CD62L and Ki67 expression. CD25 expression was induced by TCR stimulation and enhanced by the addition of rIL-2 to cell cultures. Responses by patient cells were variable as compared with responses by cells from healthy donors (Fig. 7). Interestingly, absent or very weak correlations were observed between the magnitude of CD25 induction and the magnitude of Ki67 expression among patient CD4+Vβ3+ cells irrespective of whether anti-Vβ3 Ab stimulation was performed in the presence or absence of rIL-2 (Figs. 7 and 8, A and B). Strikingly however, the induction of CD25 expression by anti-Vβ3 Ab stimulation in the absence of exogenous IL-2 correlated strongly \((r = 0.71, p = 0.05)\) with the induction of Ki67 expression in the presence of rIL-2 (Fig. 8C). This surprising observation suggests to us that the induction of CD25 by TCR engagement in the absence of rIL-2 is reflective of the efficiency of TCR activation and that this, in turn, determines the ability of cells to divide once IL-2 is provided in sufficient quantity. Analyses of TCR-activated cells from a larger patient sample that included only patients with naive CD4+Vβ3+ cell precursor frequencies in the normal range (>85% of CD4Vβ3+...
cells in CD45RO-depleted PBMC) demonstrated a similar relationship between the degree of CD62L down-modulation in the absence of rIL-2 and the degree of Ki67 expression in the presence of rIL-2 (Fig. 8D). Thus, the degree of induced CD25 expression and CD62L down-modulation that occur in the absence of rIL-2 are likely reflective of the efficiency of TCR signaling and the related events that are responsible for permitting cells to respond to IL-2 stimulation.

Discussion
Ki67 expression is first detected during late G1 phase in cells responding to mitogen (26). We observed that unlike CD25 expression and CD62L down-modulation, which could be detected within 18 h of TCR stimulation, induced Ki67 expression was not observed until 24–48 h poststimulation (data not shown), consistent with appearance in late G1 phase. Our previous experiments using bulk lymphocyte populations indicated an arrest in early G1 phase of the cell cycle in patient cells responding to TCR stimulation (21), and now by focusing on naive T cells, we have found a similar defect as indicated by the failure of patient cells to express Ki67 2 days poststimulation with anti-Vβ3 Ab.

The role of Ki67 expression in proliferation is uncertain. Failure of Ki67 expression may contribute to proliferation defects in cells from HIV-infected individuals because antisense experiments suggest a possible role for Ki67 in proliferation function (32). Alteratively, the failure to express Ki67 may reflect a more global defect in cell cycle progression that could also include a relative failure of the cells to produce IL-2 (20) or D-type cyclins (21) as well as other molecules important for cell cycle progression.

The failure of patient naive CD4+ T cells to progress through cell cycle while still expressing activation markers or down-modulating cell surface molecules such as CD62L may be related to the suboptimal TCR activation that has been observed in T cells from HIV-infected individuals (33, 34). The potential for T cells to modulate the expression of cell surface molecules is likely to require lower levels of threshold activation than more complex events such as the coordination of cell cycle progression (35–38). Thus, patient naive CD4+ T cells appear to maintain the capacity to perform certain functions requiring lower TCR activation thresholds, but are more often impaired in functions involving greater activation thresholds. Another possibility is that the most functional naive T cells are selectively lost early in HIV infection and/or there are perturbations in the naive T cell compartment that are not appreciated by using the markers of naive T cells that we have employed in this study. Thus, there may be distinct subpopulations of naive T cells that have functional defects or are “exhausted” from enhanced homeostatic proliferation and these may be enriched in HIV disease. Thus, the novel observations presented in this report suggest a model of T cell immune dysfunction in HIV-1 infection that will require confirmation through direct analyses of TCR activation processes, downstream IL-2 signaling events, and further dissection of naive T cell subtypes in HIV disease.

Our analyses of IL-2R expression (CD122 and CD132) and STAT5 phosphorylation demonstrate that responsiveness to TCR
with rIL-2 stimulation can be impaired even when the IL-2 signaling machinery is intact. It should be emphasized that the experiments described in this study represent an initial characterization involving a small number of patients so it is possible that ongoing experiments will uncover heterogeneity in these measures of IL-2 responsiveness among patient cells. Nevertheless, we provide clear examples of patient cells with normal CD122 and CD132 expression and normal P-STAT5 expression that still have Ki67 expression defects following TCR with rIL-2 stimulation. Our assessment of STAT5 activation was particularly relevant in these circumstances as suggested by studies in mice that lack both STAT5 A and B proteins. These mice, unlike mice that lack only one of the two STAT5 proteins, demonstrate marked proliferation defects in response to TCR stimulation that are not corrected by exogenous IL-2 (14). Also, the lack of proliferation function in cells from these animals was associated with poor induction of D-type cyclins, a finding analogous to our previous observations in CD4+ T cells from HIV-infected individuals that failed to respond to TCR agonistic Ab (19). Despite these parallels, we could find no evidence for STAT5 signaling impairments in patient cells using an Ab that does not distinguish between STAT5 A and B proteins.

These observations suggests that lesions may be present downstream from STAT5 signaling or that there may be interference of normal STAT5 function by truncated STAT5 expression in cells from HIV-infected patients (39). Alternatively, IL-2 signaling defects could occur in pathways other than those involving STAT5. For example, IL-2 stimulation leads to activation of the Ras-Rafmitogen-activated protein kinase pathway (40–42) as well as the phosphatidylinositol 3-kinase pathway (43–45). Thus, further experimentation will be required to determine whether these alternative pathways are selectively impaired in HIV disease.

If, as our results suggest, IL-2R expression and signaling are relatively intact among patient naive CD4+ T cells, then it may be possible that the observed defects in cell cycle progression are related to perturbations in TCR signaling pathways. At least two conceivable explanations may account for this. The degree of TCR signaling could be so poor in patient naive CD4+ T cells that even IL-2 cannot rescue function. This may explain why induced CD25 expression and CD62L down-modulation that occurs among TCR-activated cells in the absence of rIL-2 correlates with the induction of Ki67 expression following TCR ligation in the presence of IL-2. Another possible explanation stems from the concept of TCR-mediated feedback inhibition of STAT5 activation. In this model, signals from the TCR involving mitogen-activated protein kinases interfere with IL-2 induced STAT5 activation (46). Because we did not measure STAT5 in the presence of TCR stimulation, we cannot rule out this possibility. However, this model is difficult to reconcile with our studies of naive T cells because this form of negative feedback has been exclusively described in previously activated T cells that undergo TCR re-ligation.

The differential responsiveness by patient naive T cells to IL-2 in the presence of TCR activation has important implications for strategies that will use IL-2 as a vaccine adjuvant in HIV-infected patients. The results of this study suggest that this strategy may have limited effectiveness in some patients. It may therefore be prudent to develop screening mechanisms to identify patients able to respond to exogenous administration of IL-2 in the context of immunization. The results of these studies also indicate that both naive T cell depletion and also naive T cell dysfunction should be considered when developing immune restoration strategies for HIV-infected individuals.

**FIGURE 7.** Induced CD25 expression and Ki67 expression after TCR stimulation. A, CD45RO-depleted PBMC from two HIV-infected individuals (P7 and P8) and a healthy donor (C4) were stimulated with anti-Vβ3 Ab ± rIL-2 and assessed 2 days later for CD25 and CD62L expression. Histograms represent CD25 expression (x-axis) and CD62L expression (y-axis) of CD4+Vβ3+ cells. B, Induction of Ki67 (percentage Ki67+ among stimulated cells – percentage Ki67+ cells among unstimulated cells) in CD4+Vβ3+ cells is shown for these same cells.

**FIGURE 8.** TCR-induced CD25 expression and CD62L down-modulation in the absence of rIL-2 correlates with Ki67 expression in the presence of rIL-2. The relationship between induced Ki67 expression and induced CD25 expression are shown for cells incubated with anti-Vβ3 Ab alone (A), anti-Vβ3 Ab + rIL-2 (B), and anti-Vβ3 Ab alone (CD25) (C) compared with anti-Vβ3 Ab + rIL-2 (Ki67). D, The relationship between Ki67 expression and CD62L down-modulation is shown for cells from a selected group of HIV-infected subjects whose CD4+Vβ3+ cells displayed normal levels of CD62L high-expressing cells after CD45RO depletion.
Acknowledgments

We thank all the individuals who participated in these studies and we appreciate the assistance of Dr. Robert Asaad in collecting blood samples.

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