T-lymphocytes from AIDS Patients Are Unable to Synthesize Ribonucleotides de Novo in Response to Mitogenic Stimulation

IMPAIRED PYRIMIDINE RESPONSES ARE ALREADY EVIDENT AT EARLY STAGES OF HIV-1 INFECTION

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Proliferative defects have been reported at the level of DNA synthesis, even in T-lymphocytes from asymptomatic human immunodeficiency virus type-1 (HIV-1) patients. Since purine and pyrimidine ribonucleotide availability is crucial for proliferation, we compared the ability of HIV-1 and HIV-1 T-lymphocytes (>95% CD4+ and CD8+) to activate de novo biosynthetic and salvage pathways following phytohemagglutinin stimulation using [3H]-labeled precursors.

The striking abnormality already detectable in asymptomatic patients’ cells was the impaired ability of CTP, UDP-Glc, and UTP pools to expand over 72 h (44-70% of control), although ATP and GTP pools and responses were normal. In symptomatic patients, resting T-cells showed markedly reduced pyrimidine pools (53-74% of control) with no change following activation. Relatively normal ATP, GTP, and NAD pools masked the same impaired response of de novo synthesis to activation, with ATP and GTP being reduced by 50% at 48 h. Purine salvage was more active than the control in unstimulated HIV-1+ cells.

This impaired de novo synthesis in HIV-1+ T-lymphocytes severely restricts the availability of ribonucleotides for vital growth-related activities such as membrane expansion and strand break repair as well as DNA and RNA synthesis. The data indicate that resting T-lymphocytes from symptomatic patients survive through enhanced salvage, but that stimulation induces metabolic cell death, and provide an explanation for the activation-associated lymphocyte death seen in HIV-1+ T-lymphocytes.

HIV-1 infection produces a series of abnormalities that affect the hematopoietic system (1). The most striking is the profound depletion of CD4 cells, the main reservoir of the virus (2), but other alterations are seen, including general lymphopenia, granulocytopenia, and anemia accompanied by lymphoid cell depletion (1). Furthermore, unexplained diarrheas and skin problems are common complaints in infected patients (3). The hematopoietic cell precursors and basal membranes of the skin and intestine are all cells with a high rate of division. Such uniform abnormalities common to all dividing cells suggest a problem in normal mitogenesis in vivo. This is supported by the diminished capacity to produce bone marrow-derived colonies in vitro (4) and the failure of T-cells from infected patients to proliferate after appropriate stimulatory signals (5-8).

Failure of T-lymphocytes from infected patients to respond to recall antigens, mitogens, and T-cell receptor/TCR stimulation can be detected in vitro by the inability of the stimulated cells to produce interleukin-2, to incorporate tritiated thymidine, and to complete their cell cycle (5-8). Defects in proliferation are seen in both CD4 and CD8 populations in infected patients (4-8). Several explanations have been advanced for the failure of cells to proliferate after appropriate stimulatory signals. These include defects in antigen-presenting cells (6) or the lack of costimulatory molecules such as CD28, CD26 (a dipeptidyl peptidase that binds adenosine deaminase), or CD73 (a 5’-ectonucleotidase), all of which are decreased in HIV-1-infected patients (9-11). Other possibilities are that structural and regulatory proteins of the virus, such as gp120, Tat, or Nef, might interfere with lymphocyte proliferation by blocking to functional molecules (6, 12). For example, gp120 binds to the CD4 molecule and can have the same effect as anti-CD4 antibodies. Tat protein binds to the CD26 molecule (13), and Nef can bind to GTP and associate with a cellular serine kinase in T-lymphocytes (12-14).

Two main hypotheses have been put forward to account for T-cell death in HIV-1 infection. The first is programmed cell death, or apoptosis, where even unstimulated cells die within the first 24 h of culture. The nuclei of the cells display electron-dense chromatin, and the DNA within the nucleus is digested by an endogenous nuclease that cleaves chromatin between individual nucleosomes while the membrane retains its integrity (15). Apoptosis can be reversed by the addition of cytokines such as interleukin-2 and by fibroblast factors in the cultures (16). The second mechanism is the activation-associated lymphocyte death, by which cells stimulated by strong mitogenic stimuli such as phytohemagglutinin (PHA) or anti-CD3 die after 48-72 h in culture (7, 8). In contrast to cells dying from apoptosis, these cells leave the G0 phase of the cell cycle fully entering the blastic stage, defined by an increase in size and the expression of activation markers such as CD25 (interleukin-2 receptor) or Class II. These cells are nevertheless unable to complete the cell cycle, their membrane disintegrates, and they die, although no apoptotic bodies can be detected. This process cannot be reversed in cultured cells from HIV-1+ patients by the addition of interleukin-2, fibroblast factors, or supernatants of noninfected cells activated by PHA (7).

The biochemical mechanisms associated with any of these...
processes have not been defined, yet the development of effective antiviral therapy demands an understanding of how infection changes the metabolism of host lymphocytes in order to devise therapy selectively lethal to the virus. Pharmacological agents currently on trial as antiviral and immunosuppressive agents are purine and pyrimidine analogues (17). All require intact pathways of purine and pyrimidine synthesis or salvage for their activation (18). The normal human immune response also depends on the activation of housekeeping genes, which results in the stimulation of de novo synthesis (the point at which activated HIV-1-infected T-cells from asymptomatic patients reputedly perish). Altered activity has been reported for purine and pyrimidine enzymes in lymphocytes from HIV-1+ subjects, but the focus has been on disrupted cells and enzymes catalyzing ribonucleotide degradation: adenosine deaminase (19), cytidine deaminase (20), and 5'-nucleotidase (21). Experiments comparing the integrated activity of the synthetic and salvage routes in intact cultured lymphocytes (which simulate the in vivo situation most closely) are lacking.

This study stems from our earlier experiences in the inherited immunodeficiencies: adenosine deaminase and purine-nucleoside phosphorylase deficiency (21–23). The similarity between the clinical consequences of adenosine deaminase deficiency and AIDS, affecting the skin, intestine, and lung as well as the lymphoid system, is remarkable. Indeed, the first adenosine deaminase-deficient adult to be identified (two sisters in their thirties) were considered to have a syndrome of “non-HIV AIDS” prior to referral to us (23). The experiments reported here were devised to investigate whether a similar metabolic basis could be identified in purine or pyrimidine metabolism to explain the mechanisms that prevent T-lymphocytes of HIV-1+ donors from finishing their cycle. Sensitive high performance liquid chromatography (HPLC) techniques, developed to differentiate between the purine ribonucleotide and deoxyribonucleotide triphosphates that accumulate in the above disorders (21, 23), were used to measure nucleotide pools (22). In this report, we demonstrate that HIV infection seriously impairs the capacity of T-lymphocytes to synthesize the purine and pyrimidine ribonucleotide intermediates essential to enable stimulated cells to complete their cycle (43).

MATERIALS AND METHODS

Subjects—Heparinized blood was obtained from eight HIV-1-seropositive patients and eight age-matched healthy volunteers. Four patients were asymptomatic (Atlanta classification of CDCI; absolute CD4 count of >400 cells/mm3). Four were classified as CDCIVC2 (n = 2) and CDCIVC3 (n = 2) (CD4 counts of 10–50 cells/mm3). Three suffered from diarrhea and three from oral thrush at the time of the investigation. All studies outlined below were carried out in appropriate containment conditions with Ethical Committee approval.

T-lymphocyte Separation—Mononuclear cells were obtained by Ficoll-Hypaque density gradient centrifugation and incubated with 1% carbonyl iron in RPMI 1640 medium for 90 min, and the monocytes were removed with a magnet. B- and NK-cells were removed by incubation with IgM class anti-CD27 (RFB7) and anti-CD16 antibodies, followed by incubation with two rounds of baby rabbit complement at 37 °C for 45 min. The purity of the cell preparation was checked by staining with fluorescein isothiocyanate-labeled anti-CD3 antibody and was >95%.

Stimulation of T-lymphocytes—Cells were counted and resuspended at 2 × 10^6 in RPMI 1640 medium containing physiological concentrations of phosphate (1 mM) supplemented with antibiotics (penicillin/streptomycin) plus 10% heat-inactivated dilauned fetal calf serum to inactivate enzymes and to avoid the known inhibitory effects of purine and pyrimidines on de novo synthesis. The cells were incubated for 0, 1, 2, or 3 days (designated D1–D4 on the figures) in the absence or presence of PHA (5 μl). Aliquots of unstimulated and stimulated cells were removed, stained with CD25, and resuspended in 2 ml of paraformaldehyde in phosphate-buffered saline. The number of unstimulated cells and the number of blasts (CD25+ cells) in both stimulated and unstimulated cultures were measured on a Cytometer Absolute flow cytometer (ORTHO Diagnostics, High Wycombe, United Kingdom) as described previously (8) and expressed as a percentage of the original input at day 1 (Fig. 1, A–C). The number of viable healthy cells was the number of cells falling in the lymphocytic and blastic gates. Cells with small size and/or increased granularity (events outside the gates) were considered to have undergone either apoptosis or activation-associated cell death. After 72 h, the number of viable cells in unstimulated cultures was >80% in the control sample (HIV-1+) and >70% in all HIV-1+ samples, but dramatic differences were seen in the number of viable lymphocytes in the two groups after PHA stimulation. The number of viable cells recovered at 72 h from cultures of T-cells from controls ranged from 80 to 105%. In contrast, only 40–69% (mean of 57%) viable cells were recovered from the cultures of T-cells from asymptomatic patients. The recovery of viable cells in the cultures of symptomatic patients ranged from 15 to 48% (mean of 36%) from the original input (Fig. 1, D–F). More than 95% of the viable blasts expressed CD25 in all the groups.

Radioactive Tracer Studies—At 24-h intervals, cells were removed from the wells into sterile 1.5-ml Eppendorf tubes and spun at 1000 × g for 1 min. The supernatant was removed, and 100 μl of fresh medium was added plus the appropriate radiolabeled substrate ([14C]glycine, [14C]hypoxanthine, or [14C]uridine) at a final concentration of 40 μM. The tubes were then incubated in a shaking water bath for 2 h, after which the reaction was stopped by centrifugation at 1000 × g for 1 min, and the cells and medium were processed as described below.

Extraction and Assay of Ribonucleotides, Nucleosides, and Bases—The medium was removed and deproteinized by the addition of 25 μl of 40% trichloroacetic acid, and the cells were disrupted with 200 μl of 10% trichloroacetic acid. The precipitate was then removed by centrifugation for 1 min at 12,000 × g, and trichloroacetic acid in both the medium and cell extracts was removed by back-extraction with diethyl ether to a pH of 5 and frozen at −20 °C if not analyzed immediately by HPLC. The cell pellets were dissolved in 0.1 M NaOH and counted in a scintillation counter. Protein in the pellet was estimated by the method of Lowry et al. (44).

HPLC Analyses—the system used was a Waters modular system, which incorporated in-line photodiode array and radioactivity detector. 100–150 μl of T-cell extract was analyzed using the anion-exchange HPLC method reported previously for the separation and quantification of ribonucleotides and deoxyribonucleotides (22). This employed a linear phosphate buffer gradient elution system (buffer A (5 mM KH2PO4) and buffer B (0.5 M KH2PO4, 1.0 M KCl), initial pH values of 2.65 and 3.5, respectively). The pH and concentrations of buffers were adjusted when necessary to ensure adequate resolution of all nucleotides as described (22). Sensitivity was increased (1 pmol/10^6 cells) by substitution of a Phenomenex Hypersil 5 μm NH2-2 column (250 × 3.2 mm) at a flow rate of 0.5 ml/min for the Hypersil NH2 column (250 × 4.6 mm) at a flow rate of 1 ml/min, which was used previously (22). Peak identification, as well as quantification of the different ribonucleotides and deoxyribonucleotides mono-, di-, and triphosphates, was made from their characteristic UV absorption spectra and retention times compared with those of a mixture of 17 authentic standards run daily (43), with simultaneous monitoring of the rate and rate of incorporation of radiolabel into the different nucleotide pools. The clear separation of eight of the nine triphosphates, run as a separate standard, is shown in Fig. 2. Although no dNTPs were detected in our experiments, the coelution of dTTP and GTP evident in Fig. 2 should not result in spuriously elevated GTP concentrations since the expansion of dTTP pools reported in PHA-stimulated lymphocytes using indirect methods is maximally 22 pmol/10^6 cells (24).

Statistical Analysis—Statistical analyses of ribonucleotide concentration changes in response to PHA in the presence of inhibitors compared with control lymphocytes alone were performed using Student’s t test.

Chemicals—Hypoxanthine, PHA, and trichloroacetic acid were purchased from Sigma. RPMI 1640 medium, fetal calf serum, penicillin (10,000 units/ml), streptomycin (10,000 μg/ml), and 24-well plates were obtained from Life Technologies, Inc. (14C)Hypoxanthine (0.94 μCi, 53 Ci/mmol) and (14C)Glycine (0.47 μCi, 106 Ci/mmol) were purchased from Amersham plc. (14C)URidine (0.89 μCi, 56 Ci/mmol) was from DuPont NEN.
Impaired Ribonucleotide Synthesis by HIV-1-infected Lymphocytes

Results

Marked Irregularities in Pyrimidine Ribonucleotide Responses to PHA Stimulation in HIV-1+ T-lymphocytes—The first important observation in this study is that early derangements in pyrimidine ribonucleotide pools are evident even in resting T-lymphocytes (CD4+ and CD8+) from asymptomatic HIV-1+ patients, and these are exaggerated by PHA. The derangement involves both uridine and cytidine nucleotides (Fig. 3). No pyrimidine or purine deoxyribonucleotide triphosphates were detected at any time over the 72-h period. This is discussed in the accompanying paper (43). Cells from asymptomatic subjects already show a 20% depletion in UTP (Fig. 3, center bars; p < 0.05) and a blunted increment in response to PHA over 72 h (70% of control; p < 0.01). The response to PHA by CTP shows even greater impairment. Although elevated in resting cells compared with the control, CTP only doubles in 72 h (Fig. 3, center bars) relative to the 5-fold control increment (day 4 center bars; p < 0.01). The similar increase in protein content per cell in these lymphocytes from asymptomatic patients compared with that of controls (Fig. 4C), together with the parallel expansion of NAD pools (Fig. 3E) and the glycine incorporation studies (see below), is consistent with increased stimulation of the remaining viable cells (Fig. 1C). The ratio of triphosphates to diphosphates, high in most instances, also indicates the satisfactory energy state of the cells.

The noteworthy finding in symptomatic patients is that the relative normality of purine pools in resting cells masks a grossly impaired ability to respond to PHA, with adenine and guanine nucleotides actually decreasing to 50% of control at 48 h (Fig. 3, C and D, right bars; p < 0.001). The latter is consistent with the viability studies, which showed a similar decline. NAD pools in unstimulated lymphocytes from symptomatic patients also show no significant difference compared with controls, but as for pyrimidines, the response to PHA is virtually absent (Fig. 3E; p < 0.001).

Blunted Mitogenic Response of de Novo GTP Synthesis Evidence Using Radiolabeled Glycine—Pulse-labeling studies using
the purine synthetic precursor \([^{14}C]\)glycine confirm little purine synthesis de novo by resting lymphocytes from HIV-1\(^+\) or HIV-1\(^-\) subjects (Fig. 4, A and B). However, changes in the normal response are apparent immediately following PHA stimulation. While incorporation of radiolabel into ATP by cells from asymptomatic subjects resembles controls, impairment of GTP synthesis is already evident. No radiolabel was found in GDP in asymptomatic patients' cells with accumulation in IMP evident on day 3, while accumulation in GTP was half that in controls. Active protein synthesis throughout the 72 h is indicated by the steady increase shown by the cells from these asymptomatic subjects (Fig. 4C). The impaired incorporation of radiolabel into GTP is similar to that noted following PHA stimulation of HIV-1\(^-\) T-lymphocytes preincubated with the IMP dehydrogenase inhibitor ribavirin in the accompanying paper (43). The combined results in these T-lymphocytes are consistent with inhibition of DNA synthesis and blast transformation demonstrated by us previously in T-lymphocytes from asymptomatic patients (7). These earlier observations showed that while these cells can be activated and enter the G\(_i/S\) phase, they are unable to complete the cell cycle (7, 8).

By contrast, \([^{14}C]\)glycine incorporation into ATP in T-cells from symptomatic subjects was much lower. No radiolabel was incorporated into GTP by symptomatic patients' cells, with radiolabel being found only in IMP on day 4, indicating a block in the conversion of IMP to GMP (Fig. 4, A and B). These findings are in accord with the lack of any increase in protein content (Fig. 4C) and the 50% fall in ATP and GTP concentrations noted above (Fig. 3, C and D). The results indicate that the inability of symptomatic patients' cells to switch on genes activating both purine and pyrimidine de novo synthetic pathways in response to PHA induces metabolic cell death.

Salvage Pathway Activity Is Enhanced in HIV-1\(^+\) Lymphocytes—Despite the above derangements in purine synthesis, purine salvage (\([^{14}C]\)hypoxanthine incorporation into adenine and guanine nucleotides) is more active in resting T-cells from both symptomatic and asymptomatic patients (incorporation greater than control) (Fig. 5, A and B). In the asymptomatic subjects' cells, purine salvage is further stimulated by PHA. Remarkably, although the response by symptomatic patients'
cells is only slightly blunted for ATP (77% of control at 72 h; p = < 0.01), a similarly enhanced incorporation into GTP is not evident.

Pyrimidine salvage is clearly much less active than for purines in unstimulated cells from all groups, but incorporation of [14C]uridine is detectable and likewise most active in cells from symptomatic patients at zero time. The pattern of uridine incorporation into uridine ribonucleotides (Fig. 5C) following PHA stimulation in asymptomatic patients' cells is normal, but is extremely poor in symptomatic patients' cells, with virtually no conversion to cytidine ribonucleotides (Fig. 5D).

These results indicate that the ability of purine salvage to meet the requirements of resting lymphocytes from symptomatic and asymptomatic HIV-1 individuals may explain the absence of marked differences in purine ribonucleotide pools from controls. However, the reduced incorporation of hypoxanthine into GTP relative to ATP by symptomatic patients' cells suggests a block in GTP synthesis beyond the level of the de novo intermediate IMP. The enhanced salvage of uridine by resting cells from symptomatic patients is also consistent with pyrimidine starvation. Although utilization of salvaged uridine for CTP synthesis by all cells is poor, the lack of any conversion by symptomatic patients' cells indicates a block at the level of CTP synthetase.

**Fig. 3.** Mean ribonucleotide concentrations (picomoles/10^6 cells) in T-lymphocytes from controls (left bars) and asymptomatic (center bars) and symptomatic (right bars) HIV-1-seropositive patients. Shown are uridine and cytidine nucleotides (A and B), adenine and guanine nucleotides (C and D), and pyridine nucleotides (E) measured on day 1 (D1) and at 24 h (D2), 48 h (D3), and 72 h (D4) after stimulation. UDPG, UDP-Glc.
DISCUSSION

Changes occurring in ribonucleotide pools of stimulated HIV-1\textsuperscript{-} and HIV-1\textsuperscript{+} T-lymphocytes over 72 h are expressed hereon an initial cell basis to highlight the exponential expansion following PHA stimulation in control lymphocytes and the striking aberrations induced by HIV-1\textsuperscript{-} infection, namely (a) the early impairment in asymptomatic patients' cells of the normal ability of pyrimidine pools to expand in response to PHA; which is in sharp contrast to (b) the total inability of all pools in cells from symptomatic subjects to respond to PHA, with ATP and GTP pools actually showing a 50% decline over 72 h; and (c) the enhanced salvage activity in symptomatic patients' cells evident from the\textsuperscript{14}C incorporation studies, but reduced incorporation of hypoxanthine into GTP and of uridine into CTP coupled with impaired \textit{de novo} purine synthesis evident following PHA stimulation.

The important question is how do the observed metabolic derangements relate to the immunological abnormalities reviewed earlier? Although further studies are clearly needed to provide a definitive answer, the impairment of pyrimidine (and subsequently, purine and pyridine) pool expansion in HIV-1\textsuperscript{-} lymphocytes and the relationship of this sequence of events to the severity of the clinical expression have implications for several facets of the immune response other than the mere synthesis of DNA and RNA.

Considering first the findings in asymptomatic patients' cells, the selective impairment of pyrimidine responses is in marked contrast to the disproportionately greater expansion of pyrimidine pools in T-lymphocytes from healthy donors. In the accompanying paper (43), we propose that the latter could be explained by the requirement to provide the additional pyrimidine precursors essential for such growth-related activities as protein glycosylation and membrane biosynthesis. Pyrimidine salvage may be equally vital to such membrane-related processes. An additional role for dCyd kinase in the salvage of dCyd not only for DNA, but also for phosphatidylinositol synthesis via dCDP-choline and the corresponding diacylglycerol intermediate was demonstrated recently (26). The high activity of dCyd kinase (26), coupled with the low activity of cytidine deaminase, in proliferating T-lymphocytes (27) would ensure the selective channeling of (d)Cyd to (d)CTP synthesis. Consequently, the 37-fold induction of lymphocyte cytidine deaminase reportedly induced by HIV-1 infection in a human H9 cell line (20) would severely restrict salvage of both Cd and dCyd, with subsequent impairment of CTP, DNA, and phosphatidylinositol synthesis. Lymphocytes from HIV-1\textsuperscript{-}-infected subjects are known to show aberrant inositol polyphosphate metabolism (28). Such HIV-1-induced CTP depletion could have equally important implications for lipid synthesis in blasting lymphocytes. CTP depletion, accompanied by arrest of CDP-choline biosynthesis, has been noted in cell lines infected with other viruses (29). Since CDP-choline and phosphatidylcholines are active intermediates in membrane biosynthesis, derangement of both these processes might explain the HIV-1-associated membrane changes seen in asymptomatic patients' cells (9–11).

**FIG. 4.** Incorporation of radiolabeled precursor ([\textsuperscript{14}C]glycine, 40 \textmu M) of the \textit{de novo} pathway. Stimulated cells from controls and asymptomatic and symptomatic HIV-1-seropositive patients were pulse-labeled for 2 h at different intervals after stimulation: 0 h (D1), 24 h (D2), 48 (D3), and 72 h (D4). Shown is [\textsuperscript{14}C]glycine incorporation into adenine nucleotides (A) and guanine nucleotides (B). Protein content over 72 h is also shown (C).
The poor response of UTP pools and the reduced incorporation of radiolabeled glycine into GTP in asymptomatic patients' cells are additional noteworthy findings. Expansion of GTP as well as of UTP pools in stimulated lymphocytes is equally important for membrane-related processes associated with the additional growth-related demands for new glycolipid and glycoprotein synthesis, such as formation of the nucleotide dolichol phosphate-linked sugar intermediates essential for the glycosylation of adhesion molecules (30). The induction of UTP and GTP depletion is known to be a key mechanism by which hexose analogues exert their growth inhibitory or cytotoxic effects on human cells (31). The impaired expansion of UTP and UDP-Glc pools in HIV-1 T-lymphocytes from asymptomatic subjects and the poor response of GTP pools in symptomatic patients' cells would similarly influence cell-surface topography and lectin binding, thereby restricting mitogenic responses (28, 30, 32). Altered glycosylation patterns have been reported in CD45 (a molecule with tyrosine phosphatase activity) in HIV-1 cells (33).

The severe impairment evident in the PHA responses of T-lymphocytes from symptomatic patients in both de novo purine and pyrimidine synthesis, coupled with the dramatic fall in ATP and GTP concentrations and the inability to synthesize new protein, indicates that the cells are metabolically dead. These findings are similar to those reported for human blood lymphocytes incubated with deoxycoformycin and deoxyadenosine to simulate adenosine deaminase deficiency (31). The absence of responses to PHA in symptomatic patients' cells here, coupled with the changes already evident in lymphocytes from asymptomatic HIV-1 subjects discussed above (the blunted response in UTP, UDP-Glc, and particularly CTP to stimulation plus the greater reduction in de novo GTP synthesis compared with ATP), is noteworthy. The results suggest that HIV-1 infection, as well as restricting Cyd salvage, may induce a similar defect affecting glutamine-dependent reactions involving (a) the pyrimidine synthetic enzymes carbamoyl-phosphate synthetase II and CTP synthetase, (b) the purine biosynthetic enzymes formylglycinamidine synthetase and GMP synthetase, and (c) impairment of NAD synthetase.

Inhibition of NAD synthetase in HIV-1 cells could provide an additional explanation for some of the abnormalities observed, if as recent studies suggest, NAD depletion has implications for the normal repair of DNA strand breaks in dividing cells.

Fig. 5. Incorporation of radiolabeled precursors (40 μM) of the purine salvage ([14C]hypoxanthine) and pyrimidine salvage ([14C]uridine) pathways. Stimulated cells from controls and asymptomatic and symptomatic HIV-1-seropositive patients were pulse-labeled for 2 h at different intervals after stimulation: 0 h (D1), 24 h (D2), 48 h (D3), and 72 h (D4). Shown is [14C]hypoxanthine incorporation into adenine nucleotides (A) and guanine nucleotides (B) and [14C]uridine incorporation into uridine nucleotides (C) and cytidine nucleotides (D). UDPG, UDP-Glc.
rather than resting lymphocytes (38). NAD depletion (not evident in resting HIV-1 lymphocytes from either asymptomatic or symptomatic patients in this study, presumably due to enhanced purine salvage) has been proposed to explain the lymphoprotection to unstimulated lymphocytes caused by the accumulation of dAdo in inherited adenosine deaminase deficiency (35, 39). NAD is an essential substrate for chromatin-associated poly(ADP-ribose) synthetase that is activated by DNA strand breaks (38–41). The triggering of programed cell death by the dAdo analogue 2-chlorodeoxyadenosine, recently developed as an immunosuppressive and antineoplastic agent, has been related to the accumulation of DNA strand breaks in both nondividing lymphocytes and monocytes, with subsequent NAD depletion, inhibition of RNA synthesis, and eventual cell lysis (41, 42). In vitro studies demonstrated that the initial DNA damage triggered the activation of a Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-dependent endonuclease, resulting in an oligonucleosomal fragmentation pattern and morphological changes typical of apoptosis (42). The NAD depletion evident in stimulated cells from the symptomatic patients’ cells here, coupled with the severe ATP depletion, would provide an additional explanation for the mechanism by which HIV-1 infection eventually induces lymphopenia and metabolic cell death. Whether metabolic cell death and apoptosis are related in any way requires further study.

The effect of HIV-1 infection on several metabolic aspects of lymphocyte proliferation demonstrated here could, as in adenosine deaminase deficiency, involve all cells undergoing active cell division and thereby explain the clinical findings in HIV-1-infected subjects relating to other cells of the hematopoietic system and involving cells of the skin and intestine as well. This study provides a metabolic explanation for the activation-associated cell death seen in T-lymphocytes from HIV-1-infected patients. The imbalance between the impaired de novo nucleotide synthesis and overstimulation of the salvage pathway explains the discrepancies in the number of viable cells from non-stimulated versus mitogen stimulated cells after 72 h of culture. While unstimulated cells can replenish their nucleotide content through the salvage pathway, this is insufficient to enable stimulated cells to complete the cell cycle. Stimulated T-lymphocytes from asymptomatic patients and possibly some cells from symptomatic patients can initiate de novo synthesis to supply enough nucleotides to enable the cells to reach the blastic stage, but the nucleotide imbalance and membrane changes induced by the impaired pyrimidine nucleotide expansion make it impossible for these cells to complete the cell cycle. Such discrepancies could also explain why cells from HIV-1-infected patients are more susceptible to activation-associated cell death when stimulated by a strong mitogen, such as PHA, than when stimulated by milder mitogenic stimuli, such as pokeweed mitogen or protein A. Our data also explain how HIV-1 infection by inducing cytidine deaminase impairs pyrimidine salvage, which may be significant for nondividing cells as well (20, 27).

Further studies are in progress to establish the level(s) at which HIV infection induces the observed irregularities and to pinpoint the enzyme(s) affected. Such knowledge has important implications for the design of new approaches to therapy.

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T-lymphocytes from AIDS Patients Are Unable to Synthesize Ribonucleotides de Novo in Response to Mitogenic Stimulation: IMPAIRED PYRIMIDINE RESPONSES ARE ALREADY EVIDENT AT EARLY STAGES OF HIV-1 INFECTION

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