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High levels of heterodisperse RNAs accumulate in T cells infected with human immunodeficiency virus and in normal thymocytes

(molecular subtraction/aberrant RNA/repetitive sequences/cytopathic effects/acquired immunodeficiency syndrome)

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ABSTRACT **Infection by human immunodeficiency virus (HIV-1) of a human T leukemia cell line (HUT 78) leads to a rapid accumulation at an elevated level (10- to 20-fold) of heterodisperse RNAs, revealed by their containing repetitive sequences. Sequence data indicate that the repetitive elements (e.g., *Alu*) are associated with transcripts with no apparent long open reading frame. In contrast, a large increase of such heterodisperse RNAs is not seen in T lymphocytes activated by phytohemagglutinin or in a variety of leukemic cell lines. Examination of several cellular messages showed that the levels of some of their "fully processed" transcripts are reduced late after infection of HUT 78 cells, indicating that the levels of some mRNAs may decrease. Surprisingly, similar heterodisperse transcripts are also seen in great abundance in normal fresh thymocytes. It is possible that in HIV-infected T lymphocytes, the accumulation of high levels of such aberrant RNAs may directly or indirectly contribute to the death of HIV-infected T cells.**

The etiological agent for acquired immunodeficiency syndrome (AIDS) has been shown to be a retrovirus, the human immunodeficiency virus (HIV) (1, 2). The course of the disease is characterized by a gradual decrease in immune function, leading to a complete failure to mount T cell-dependent responses to bacterial and viral infections (3, 4). At the cellular level, the deterioration of the immune system is reflected in a dramatic reduction in the number of helper T lymphocytes and in the ratio of helper to cytotoxic/suppressor T cells. Thus, it would appear that the principal cause of immune dysfunction is the depletion of the helper T-cell compartment (3, 4).

Studies using cultured T-cell lines and peripheral T lymphocytes infected with HIV *in vitro* have shown that HIV infection produces dramatic cytopathic effects in CD4-carrying T lymphocytes (3–5). CD4, a helper T lymphocyte-associated surface protein, has been shown to be the viral receptor (6–9), thereby providing an explanation for the target specificity of HIV. HIV can directly infect $CD4^+$ lymphocytes, which could lead to a major disruption of the immune system by depletion of a population that is a crucial part of the immune system.

The cellular basis by which HIV cause these cytopathic effects is not known. It has been suggested that the primary way by which cell death occurs is via an interaction between the viral glycoprotein, gp120, and the HIV receptor, CD4. This may lead to the formation of syncytia and subsequent generalized cell fusion, causing death (10). Other changes that may affect the HIV-infected lymphocytes include the presence of specific HIV-related antigens (11) and a possible cytotoxic effect by killer lymphocytes directed against viral components (12, 13). On the other hand, it is possible that HIV infection interferes with other fundamental host-cell functions, resulting in compromised T lymphocytes, but this possibility has not been investigated thoroughly to date. In this study, we show that HIV infection of a T-cell line can result in the accumulation of high levels of large molecular weight heterodisperse RNAs (RNAs containing repetitive elements) suggesting that HIV infection may interfere with the normal synthesis, degradation, transport, or processing of RNA. We speculate that this deregulation may contribute to the death of T cells infected with HIV.

MATERIALS AND METHODS

Cells and Cell Culture. The thymic leukemia cell lines and their properties have been described (14). Cells were cultured in a RPMI 1640 medium containing 10% (vol/vol) fetal calf serum, 20 mM L-glutamine, and antibiotics.

Infection of HUT 78 Cells by HIV. B40 and B117 strains of HIV (15) were passaged once in peripheral blood mononuclear cells, and the supernatant culture fluid was filtered (0.45-μm millipore) and used to infect T-cell HUT 78 cells. Virus containing reverse transcriptase activity that catalyzed the incorporation of 70,000 cpm of [^{32}P]dCMP into CCl_3COOH-insoluble material was added to 10^6 cells and incubated for 1 hr at 37°C, followed by one cycle of centrifugation to remove excess virus. Thereafter, cells were resuspended in RPMI 1640 medium containing 10% fetal calf serum, 2 μg of Polybrene per ml, and antibiotics.

cDNA Synthesis and Differential Screening. At zero time and at 2, 3, or 4 days after infection by HIV, cells were harvested, and the RNA was extracted as described (16). Complementary DNA was synthesized by using oligo(dT) as a primer and total RNA from cells 3 days after infection with HIV (17). Two million independent cDNA clones were made. First-strand cDNA was also hybridized to poly(A)-enriched RNA from uninfected HUT 78 cells to a C_{rt} value (an initial concentration of RNA) of 1200 mol·liter^{-1}·sec before passage through a hydroxylapatite column to separate the hybridized cDNA from the unhybridized cDNA. Using this [^{32}P]dCTP-labeled hybridized first-strand cDNA and first-strand cDNA from uninfected HUT 78 cellular RNA as probes, we screened 50,000 cDNA clones harvested 3 days after HIV infection for cDNA clones preferentially expressed in HIV-infected HUT 78 cells (17, 18).

RNA Blot-Hybridization and Dot-Blot Analysis. RNA was extracted from cell lines or HIV-infected cells in the presence of guanidine hydrochloride (16), and 10 μg of RNA was treated with glyoxal, electrophoresed through a 1% agarose gel in a 10 mM sodium phosphate buffer (pH 7.0), and transferred to GeneScreen*Plus* (New England Nuclear) according to the manufacturer's directions. For dot-blot analysis, various concentrations of RNAs were dotted on the same filters. Filters were hybridized overnight at 65°C in 5×

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Abbreviation: HIV, human immunodeficiency virus.

SSC ($1\times$ = 0.15 M NaCl/0.015 M sodium citrate, pH 7) containing $5\times$ Denhardt's solution ($1\times$ = 0.02% polyvinylpyrrolidone/0.02% bovine serum albumin/0.02% Ficoll), 10% dextran sulfate, 1% sodium dodecyl sulfate, and 100 μg of salmon sperm DNA per ml and were washed in $3\times$ SSC at 65°C. Molecular hybridization was done with ^{32}P-labeled nick-translated probes at 10^6 cpm/ml (19).

DNA Sequencing. DNA sequences of the cDNAs were obtained by using Sanger's method (20). Details of the procedure used have been reported (17, 18).

Primer Extension. Primer extension of HIV-infected and uninfected RNA was carried out with a primer homologous to the *Alu* sequences. Ten micrograms of RNAs from HUT 78 cells both before infection and 2 days after infection with HIV, from fresh human thymocytes or from peripheral blood T lymphocytes stimulated by phytohemagglutinin, were hybridized to a 36-mer oligonucleotide homologous to a consensus sequence at the 3′ end of an *Alu* sequence (CCAGGCTGGAGTGCAGTGGCGCGATCTCGGCTCACT). One hundred units of reverse transcriptase was added, and first-strand cDNAs were synthesized by using ^{32}P-labeled triphosphates. The products were electrophoresed through an 8% polyacrylamide gel.

RESULTS

Messages Enhanced in T Cells by HIV Infection. To determine whether certain genes are preferentially expressed after HIV infection of T cells, we attempted to clone cDNAs corresponding to messages transcribed in abundance after HIV infection but expressed at low or undetectable levels prior to infection. Techniques of subtraction hybridization and differential screening were as described (17, 18). Cells from the T-cell line HUT 78 were infected *in vitro* with high titers of HIV. Two, 3, and 4 days later, RNA was extracted from these infected cells, and a cDNA library was constructed with RNA obtained on postinfection days 3 and 4. Cytopathic effects were observed 3 days after HIV infection. A library of 2×10^6 independent clones (from an unamplified library) was obtained. Fifty thousand cDNA clones were screened either with radioactively labeled cDNA derived from RNA from infected HUT 78 cells [after hybridization with poly(A)$^+$ RNA from uninfected cells and removal of shared sequences by hydroxyapatite columns] or with cDNA synthesized from uninfected HUT 78 cells (17, 18). Using this procedure, we isolated 12 cDNAs (KA3, KA7, KA8, KA12, KA18, KA24, KA29, KA32, KA33, KA40, KA44, and KA50) that are preferentially expressed in HIV-infected HUT 78 cells. The lengths of these cDNAs are about 1–5 kilobases (kb). Examples of a dot-blot analysis with RNAs from uninfected HUT 78 cells, HIV-infected HUT 78 cells (days 2, 3, and 4), and uninfected Jurkat and RPMI 3638 B cells are shown in Fig. 1 *Upper*. The dot-blot analysis shows an approximate 10- to 20-fold increase after infection in the number of transcripts corresponding to one of these cDNAs (Fig. 1 *Lower*).

To determine if these 12 cDNAs represent one or more classes of messages induced by HIV infection, the clones were hybridized to each other by using Southern blot analysis techniques coupled with an analysis using several different restriction enzymes. It was found that, although certain segments of each of the cDNAs shared homology with each other, sequences unique to the 12 clones were also found (data not shown).

HIV-Induced RNAs Contain Repetitive Sequences. To identify these messages as well as sequences associated with each of these 12 cDNAs, blot-hybridization analyses of human DNA and RNA derived from control HUT 78 cells and from cells infected with HIV were performed. Results indicated that each of these 12 cDNA clones hybridized to multiple bands in human DNA to an extent that suggested the presence of highly repetitive sequences in the cDNAs (data not shown). Using several of these HIV-enhanced cDNAs as individual probes, we detected no discrete bands in RNA from either uninfected or HIV-infected HUT 78 cells; instead, hybridization to RNAs of all sizes was detected (Fig. 2*a*). In the infected cells (day 3 after infection), abundant amounts of high molecular weight transcripts (from <1 to >10 kb) were found. In this experiment, cytopathic effects were observed 3 days after infection. After treatment with DNase (in the presence of RNase inhibitors), the large RNA transcripts

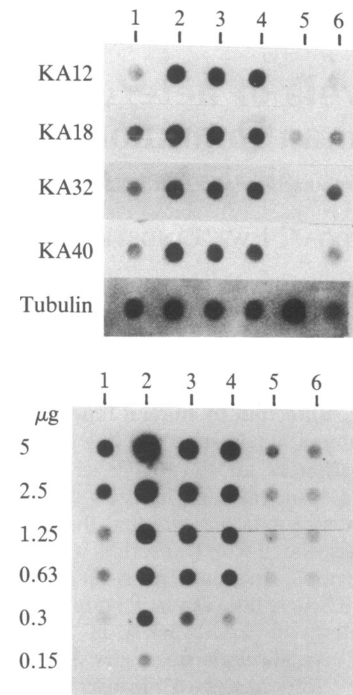

FIG. 1. (*Upper*) Expression of messages enhanced after HIV infection of HUT 78 cells. Twelve cDNAs corresponding to messages enhanced after HIV infection of HUT 78 cells were isolated. A dot-blot analysis is shown using four such clones (KA12, KA18, KA32, or KA40) as probes and RNAs from uninfected HUT 78 cells (lane 1); from infected HUT-78 cells on days 2 (lane 2), 3 (lane 3), and 4 (lane 4) after infection; from T-cell line Jurkat (lane 5); and from B-cell line RPMI 3638 (lane 6). One microgram of total RNA from these cells was applied to nitrocellulose paper and hybridized by using [^{32}P]dCTP-labeled cDNAs from these clones. A cDNA clone of human tubulin (a gift from N. Miyamoto, Ontario Cancer Institute) was used as the control. (*Lower*) Dot-blot analysis of RNA from HIV-infected cells using a HIV-enhanced probe KA3. RNAs were extracted from HUT 78 cells on days 0 (uninfected), 2, 3, and 4 after infection with HIV and are identified as in *Upper*. RNAs from T-cell line Jurkat (lane 5) and B-cell line RPMI 3638 (lane 6) were included. The dot-blot analysis shows an approximate 10- to 20-fold (estimated by eye) increase in the amount of transcript in the infected cells.

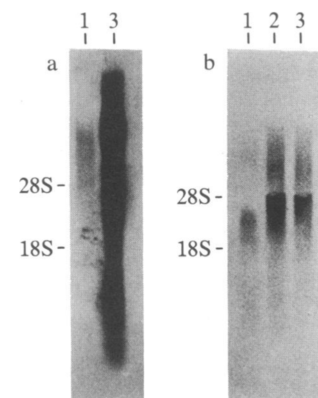

FIG. 2. RNA blot-hybridization analysis of RNA from HUT 78 cells before (lanes 1) or 2 (lane 2) or 3 (lanes 3) days after infection with HIV. RNAs from HIV-infected or uninfected HUT 78 cells were analyzed by blot hybridization. (*a*) Filters were hybridized with KA12 as probe. (*b*) A member of the *Kpn* 1 repetitive gene family (21), PBK1.2, was used as probe.

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                 ←  Alu ·  Alu →
  1  GGCTGCAGTGAGCCATGATCACGCCACTGCACTCCAGCCTGGGCAACAGAGCAAGACC
 61  GCCTCAAAAAAAAAAAAAAAAAAA|GGCTGGGACAGTGGCTCATGCCTGCAATCCCAGCAC
121  TGGAAGGCCGAGGCAGGTGGATCACCTGAGGTCGGGAGTTTGAGGCCAGGCTGGCAAC
181  GGTAAAACTCCATCTCTACTAAAAATACAAAAATTAGTCACGCATGGTGGCGCACGCT
241  AATCCCAGCTACTGGGGAGGCTGAGGTGGGAGAATTGCTTGAACCCAGGAGGTGGAGG
301  GCAGTGAGCTGAGATTGTGCCACTGCACTACAGCCTGGACAACAAGCAAGACTCTGTC
          ←Alu
361  AAAAAAAAAAAAAAAAAAAA|GAGAAGAGAAGAGAGCCCTGTGCAAGGTGGGAAAAAAATG
421  AAAACTATAACTAAGAATTTAATATCTCACCTTTCTTTTTTTCTTAACAACAACAAAA
481  AGATTTTTAGAAGTAATTGAATGAATTTCAAAGGTTTTGGTAGACATGGCCATCTGCT
541  TCAGTTCAGGCTGCAGGTGTAACTTCAGCTATATCCCTTCCTGCCGCTGAAAATGGTG
601  TGTTCAGGTGTGGGAAGATCAGGCAGCTGGCTTTGGCAGAGCAAGAGGCTTCAGAGCA
661  AGCTAGCATATTTAGATGTATATTATTATATCATGTCACTCATAGTGAGTTTCTGTAA
721  ATCCTTGTAATAACCTAATAACCAAATCCATTTTCTAAGCATTATCCAAAATGCTTTC
781  TCATACTGTCCCACTGAGCCTTTGCAACAGCTCTGAGAGGTGGGCTAATAATATTAAC
841  CATTTTACAAAGAAAGCTGTGGAAGTGAGTTGCTTTCAGGTCTCACAGCGCAGGAGAG
901  AGAGCTGGGGCTCCAAGTTAGAGCCAGCTTCCTTTGAATTC
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  1  ACTAGAATAGGAATAAAATGATTAAACAATATGTATTTAATTTGCTAGCCAATGTCAT
 61  TTAATGGTGAAAAGCTAGGAATACTGCTTGTAAAGCCAGGGCTAGAAATAATGCCTGC
121  TCACAACTACTACTTAACATTGTTCTGAGGTCTAGCCTTGTGAAGTAATTAGAAAGAA
181  CTAAAATTAATGAAGAAATATAGATATTAAAAAGACAGTCACAAAAATTACTATAGGA
241  ATATAATTGCATACCTGGAAAACAGAAGCAGCTTTTTTAAAAAGCTAAAGTGTACTAT
                                              Alu →
301  TTCAGTAAGGTGTCCACTGATAAAAATTATATAAAAA|GGCCAGGCACAGTGGCTCAAG
361  TTAATCCCAGCACTTTGGTAGGCTGAGGCAGGCGGATC
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FIG. 3. Nucleotide sequence of two cDNA clones preferentially expressed in HIV-infected HUT 78 cells. Two of the cDNA clones obtained by molecular subtraction and differential screening between HIV-infected and uninfected HUT 78 cells were sequenced by the Sanger M13 dideoxy method (20). The *Alu* repetitive sequences are marked. (*Upper*) Complete sequence of KA12. (*Lower*) Partial sequence of KA32.

remained (unpublished data), eliminating the possibility that hybridization was due to contaminating DNA in the RNA samples. Our results suggest that the sequences that become abundant as a result of HIV infection of HUT 78 cells contain repetitive sequences and are found in RNAs with low to very high molecular weights.

Sequence of the Message Enhanced by HIV Infection. To identify the messages stimulated by HIV infection of HUT 78 cells, the entire nucleotide sequence of one cDNA clone (KA12) and the partial nucleotide sequence of another independent clone (KA32) were determined (Fig. 3 *Upper* and *Lower*, respectively).[§] The shared sequences are part of the *Alu* family of repetitive sequences. Hybridization studies showed that only the *Alu* segments of each of these 12 clones share sequences with each other, while the non-*Alu* parts of the messages from these cDNAs are unique (data not shown). The *Alu* sequences are associated with different nucleotide sequences in each clone and are in different positions (for example, in clone KA12, the *Alu* sequences are situated at the 5′ end, while in clone KA32, they are in the middle of the sequence). The non-*Alu* sequences in both clones KA12 and KA32 contained no long open reading frames [the longest of which is about 250 base pairs (bp)]. These results suggest that infection of HUT 78 cells with HIV leads to an increase in accumulation of RNA containing *Alu* repetitive sequences.

HIV-Infected T Cells Accumulate High Levels of Heterodisperse RNA. The possibility that RNA polymerase III was responsible for the abundant levels of *Alu*-containing messages (22) was tested by analyzing the position of the *Alu* sequences in relation to the transcription initiation points. Primer extension analysis with RNA from HIV-infected cells and an oligonucleotide sequence complementary to either the positive or the negative strand of the *Alu* consensus sequence was performed. Using an oligonucleotide complementary to 3′ end of the *Alu* sequence as a primer, we found no increase in the level of 300-bp DNA unit (one *Alu* sequence) in the infected cells, which suggest that these RNAs did not initiate in these *Alu* segments (Fig. 4). In fact, the level of the 300-bp *Alu* sequence decreased after infection. Thus, it is likely that the abundant level of *Alu*-containing RNA in HIV-infected cells was not due to an increased RNA polymerase III transcription of *Alu* sequences.

To examine if these heterodisperse RNAs also carry other repetitive sequences, a member of the *Kpn* 1 family of repetitive sequences (21, 22) was also used as a probe to

[§]The sequence for clone KA12 reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03812).

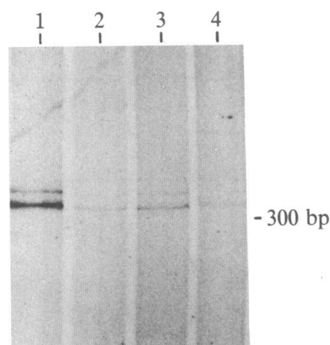

FIG. 4. Primer extension of HIV-infected and uninfected RNA by using a primer homologous to the 3′ end of the consensus *Alu* sequence. Lanes: 1, uninfected HUT 78 cells; 2, infected HUT 78 cells; 3, thymocytes; 4, peripheral blood T lymphocytes.

investigate RNAs from HIV-infected or uninfected cells in blot-hybridization analysis. High molecular weight RNA containing these *Kpn* I sequences was found in abundance 2 or 3 days after the HUT 78 cells were infected with HIV (Fig. 2*b*).

To evaluate if the heterodisperse RNAs that accumulated in HIV-infected cells affect the level of mRNA of some cellular genes, we performed RNA blot-hybridization analysis to determine the message sizes and expression levels of

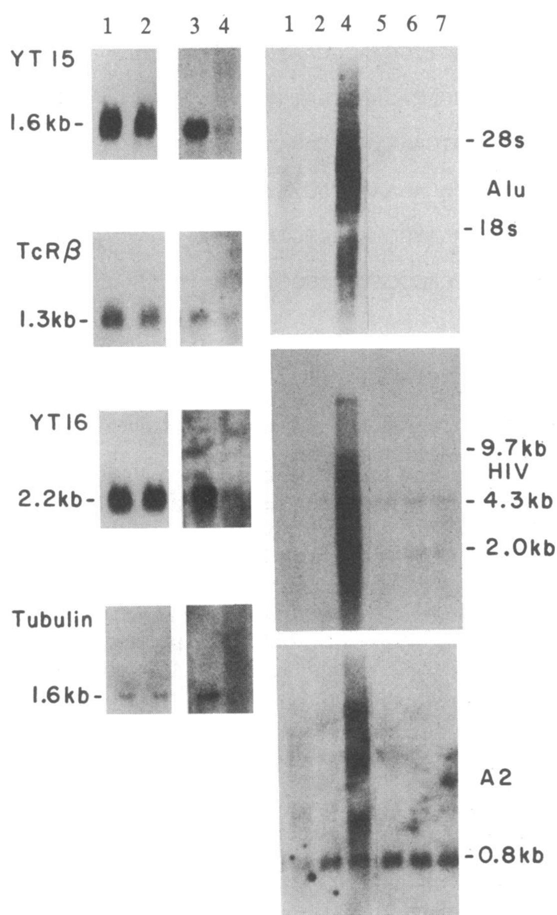

FIG. 5. Blot-hybridization analysis of total RNA from uninfected or HIV-infected HUT 78 cells (lanes 1–4) or leukemia cell lines [MOLT-3 (lane 5), MOLT-4 (lane 6), and P30/OKUBO (lane 7)] by using a variety of cDNAs or HIV as probes. RNA was extracted from uninfected (lane 1) or early (day 2) (lanes 2 and 3) and late (day 4) (lanes 4) infected HUT 78 cells. Total RNA was analyzed with cDNA probes derived from messages for the T-cell receptor β chain (TcRβ), tubulin, YT15, YT16, KA32 (*Alu* containing), or A2 or from HIV.

several cellular transcripts from uninfected and HIV-infected HUT 78 cells (Fig. 5). In this infection, cytopathic effects were observed 5–6 days after infection. We used probes derived from cDNAs for the T-cell-antigen receptor β chain, human tubulin, YT15 and YT16, (two T-cell-specific mRNAs), KA32, HIV, and A2 (a cellular message enhanced in cells from patients with adult T-cell leukemia). Results show that, although the levels of all of these transcripts remained unchanged early (day 2) after infection with HIV, the levels of many of the mRNA (YT15, YT16, T-cell receptor β chain, and tubulin) were greatly reduced late (day 4) after infection. This is contrasted by the high level of HIV messages and transcripts with *Alu* sequences (Fig. 5). In these experiments, high levels of heterodisperse RNA could be detected late after infection. The level of the "fully processed" transcripts from A2, on the other hand, remained unchanged, although discrete A2 transcripts with higher molecular weights were also evident.

High Molecular Weight RNA in Thymocytes. We next searched for other situations of accumulations of heterodisperse RNAs, using repetitive sequences as probes. When fresh thymocytes from two different thymuses were examined, they were found to contain high levels of these heterodisperse RNAs (Fig. 6). In comparison, however, only low levels of such RNAs were found in phytohemagglutinin-stimulated peripheral T lymphocytes and in other T- and B-cell lines—namely, RPMI 3638 (Fig. 1), Jurkat (Fig. 1), MOLT-3 (Fig. 5), MOLT-4 (Fig. 5), P30/OKUBO (Fig. 5), and RPMI 1788 (data not shown).

DISCUSSION

Our data indicate that cells from a T-cell line, HUT 78, upon infection with HIV, produce high levels of large molecular weight transcripts containing sequences that belong to the *Alu* family, a group of repetitive human sequences. All 12 cDNAs cloned from a subtractive library, differentially screened with RNA from HIV-infected and uninfected HUT 78 cells, contain *Alu* as part of their sequences. Molecular subtraction has been shown to be efficient in isolation of transcripts expressed in one type of cell as opposed to those expressed in another cell type and has been used successfully in the isolation of several genes (17–19, 23), the most notable of which are the T-cell receptor genes (17, 18, 23). The findings that all of the cDNAs isolated in this manner contained *Alu* sequences and that two of these cDNAs (the only ones sequenced so far) contained no long translational open reading frames that HIV infection does not induce readily detectable functional cellular messages specific for HIV-infected cells.

Although high levels of specific genes do not appear to be induced by HIV infection of these T cells, a dramatic increase in cellular transcripts containing *Alu* sequences can be easily detected. The reason(s) for this rapid accumulation of heterodisperse RNA is not known. One possibility is that the

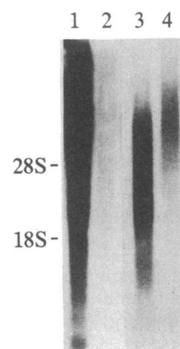

FIG. 6. Blot-hybridization analyses of RNAs extracted from two different preparations of fresh thymocytes (lanes 1 and 3) or phytohemagglutinin-stimulated peripheral blood T lymphocytes (lanes 2 and 4) with KA12 (a gift from J. Maio, Albert Einstein School of Medicine) as probe.

HIV infection leads to active transcription of RNA containing *Alu* sequences (by RNA polymerase III) (22). This possibility was investigated, and our results tend to rule out this possibility. In primer extension analysis, the levels of *Alu* transcript units of 300 bp did not increase in the infected cells. In addition to the *Alu* sequences, at least one other type of repetitive sequence family (*Kpn* I family) (21, 22) was also found to be elevated in the HIV-infected cells. Thus, these repetitive sequences are probably present as part of the introns or nontranslated sequences of certain actively transcribing genes. Other possible explanations for the change in RNA pattern in HIV-infected T cells could be (*i*) active RNA synthesis resulting from nonspecific transcription, (*ii*) an altered rate of transport of RNA from the nucleus to the cytoplasm, or (*iii*) change in the rates of degradation of unprocessed or aberrant RNA.

Irrespective of the mechanism(s), one consequence of the abnormal changes of RNA pattern in HIV-infected cells could be the reduction of the level of cellular mRNA. A survey of some cellular and T cell-specific messages revealed that this was indeed the case. The levels of the fully processed transcripts for several of these mRNA decreased late after infection. For example, while the amounts of high molecular weight RNAs (as detected through the use of *Alu* sequences as probes) and the HIV-specific sequences increased late after infection, the levels of messages of YT15, T-cell receptor β chain, YT16, and tubulin were drastically reduced. In at least one cellular message (A2), the "fully processed messages" maintained a normal level. Furthermore, several additional higher molecular transcripts of this gene were also detected. The lower level of some but not all "messages" and the presence of several large-size transcripts (at least of the *A2* gene) support the hypothesis that the abnormality may affect only some RNA species late after infection with HIV. These phenomena may explain the decreased levels of CD4 mRNA and surface CD4 in HIV-infected T cells (24). It is not difficult to conclude that the deprivation of HIV-infected T cells of significant amounts of many of the mRNAs can affect their physiological states.

Curiously, we also have found that fresh thymocytes harbor an abundant amount of these heterodisperse RNAs. The high levels of heterodisperse RNAs in these thymocytes cannot be explained purely by the rapid proliferation of these cells, as T lymphocytes stimulated by phytohemagglutinin did not contain high levels of these messages. Other rapidly proliferating cells, such as Epstein–Barr virus-transformed B lymphocytes (RPMI 1788 and RPMI 3638) or T-cell leukemia lines (Jurkat, MOLT-3, MOLT-4, and P30/OKUBO) also contain low amounts of these RNAs.

The mechanism by which the viral infection induces such change in the pattern of RNA is unknown, and it is not clear whether the "virulence" or replication potential of the substrains of HIV used (15) may affect the levels of these abnormal heterodisperse RNAs. It is possible that one or a combination of the viral products directly affect the accumulation of such heterodisperse RNA. A likely candidate would be the *art/trs* genes that are known to interfere with viral RNA processing (25–27).

At the present time, we do not know the significance of the accumulation of high levels of these heterodisperse RNAs in HIV-infected T cells, nor do we know the mechanism by which viral infection induces these effects. It is tempting to speculate, however, that the change in the pattern of cellular RNA may be involved in or contribute to the cytopathic effects seen in HIV-infected T cells. Alternatively, the activation or suppression of certain cellular genes may precipitate such abnormal changes. Whether these phenomena contribute to, or by themselves adversely affect, the $CD4^+$ T lymphocytes and thus indirectly alter the course of AIDS is not known. It is likely, however, that a combination of some of these factors—including the interaction between CD4 and the viral coat protein gp120, leading to syncytia formation (10); autoimmune reactions, possibly due to the presence of cytotoxic T cells directed against viral components (12, 13); specific antigens in HIV-infected cells (11); and the effects of accumulation of high levels of heterodisperse RNAs (this manuscript)—may contribute to the overall picture.

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