Gibbon Ape Leukaemia Virus RNA in Leukaemic T-Lymphoid Cell Lines: Expression of a Novel RNA Transcript

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SUMMARY

Fibroblast cell lines infected in vitro with different strains of gibbon ape leukaemia virus or the related woolly monkey virus (SSAV) synthesized two RNA species of approximately 8-4 kb and 2-9 kb. The former, a complete RNA, represents the gag-pol mRNA, while the latter is a spliced transcript lacking gag and pol, and represents the env mRNA. In contrast, RNA from one T-lymphoid cell line derived from a gibbon ape T-lymphocytic leukaemia (UCD-144) expressed a viral mRNA in addition to gag-pol and env mRNA. This RNA is 6-4 kb and lacks at least 3-0 kb of sequences derived from the internal region of the viral genome, including most or all of the pol gene. These data, as well as data from Southern blots of UCD-144 DNA, suggest that the 6-4 kb mRNA could represent a transcript from a defective recombinant provirus and may contain cell-derived sequences.

The gibbon ape leukaemia viruses (GaLV) and the woolly monkey virus (SSAV) form a closely related group of infectious retroviruses occurring naturally in gibbon apes. Several types of GaLV have been aetiologically implicated in leukaemia. GaLV-SEATO (SEATO strain) (Kawakami & Buckley, 1974) causes myeloid leukaemia when inoculated into gibbon apes (Kawakami et al., 1980). GaLV-H (Hall's Island strain) (Gallo et al., 1978) and GaLV-SF (San Francisco strain) (Kawakami et al., 1972) have been identified by seroepidemiology as the primary agent in lymphocytic leukaemia in gibbon apes (Kawakami et al., 1973; Krakower et al., 1978).

Several different possible mechanisms of induction of haematopoietic neoplasms by retroviruses have been described. Acutely transforming viruses contain transduced cellular genes and directly cause transformation when re-introduced into susceptible cells with the aid of helper viruses. Generation of these types of viruses occurs outside the laboratory in cats (for review, see Hardy, 1980) and also apparently occurred after infection of a woolly monkey with a GaLV-related virus from an infected gibbon (Theilen et al., 1971; Wolfe et al., 1971). Some retroviruses which are oncogenic but do not directly transform cells, such as avian leukemia viruses and mouse mammary tumour virus, have been shown to integrate into specific areas of the host genome and cause inappropriate transcription of cellular onc genes (Hayward et al., 1981; Neel et al., 1981; Nusse & Varmus, 1982; Payne et al., 1982). This process is thought to contribute critically to transformation and has been termed downstream promotion or transcriptional enhancement. In the case of the avian viruses, chimeric RNA transcripts containing both viral and cellular sequences have been identified. In order to understand better the relationship of GaLV to leukaemia in gibbon apes, we have characterized viral transcripts in several GaLV-infected gibbon ape leukaemic cell lines.

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Fig. 1. Restriction endonuclease maps of two GaLV proviruses and a schematic representation of the regions of the 6-4 kb GaLV-SF viral transcript which contains viral sequences. The GaLV-SF provirus in (a) corresponds to the GaLV-SF clone of Scott et al. (1981). (b) shows the content of viral sequences in the 6-4 kb GaLV-SF transcript present in the UCD-144 cell line. (c) shows the map of the GaLV-SEATO provirus and the restriction enzyme fragments which correspond to the five subclones used to characterize viral transcripts. E, EcoRl; Sa, SalI; P, PstI. (A) to (E) indicate GAS-A to GAS-E probes respectively (see text).

The 6G-1 cell line, infected with and producing GaLV-H, is a T-lymphoid line derived from a gibbon ape with lymphoid leukaemia (Gallo et al., 1978). The UCD-144 cell line is another T-lymphoid leukaemic cell line established from a gibbon ape with lymphoid leukaemia, and is infected with and producing GaLV-SF, which is related to but distinct from GaLV-H (Kawakami et al., 1972). Bat lung fibroblasts (CCL-88) infected with GaLV-SEATO (Kawakami & Buckley, 1974) and GaLV-Br (Todaro et al., 1975) and human fibroblasts (A204) infected with SSAV were also used in these studies.

The DNA clone λ-GAS-2 has been previously described (Gelmann et al., 1982) and contains a permuted complete copy of unintegrated viral DNA of GaLV-SEATO, including two large terminal repeat (LTR) sequences. The DNA clone λ-GAS-1 is similar but differs by a SalI site and contains only one LTR. Derived subclones include a permuted complete LTR containing approximately 500 bp between the PstI sites in the adjacent LTRs from pGAS-2, a U3–R fragment between the EcoRI and PstI LTR sites (GAS-A), a 3' fragment including the env region between the PstI site at the 5' end of the LTR and an EcoRI site (GAS-B), a pol-containing SalI fragment (GAS-C), a fragment between SalI and EcoRI in the gag–pol junction region (GAS-D), and an EcoRI fragment containing R, U5 and gag sequences (GAS-E), all from pGAS-1. The composition of these probes is summarized in Fig. 1. DNA and RNA purification and Southern and Northern blot analyses have been previously described (Reitz et al., 1984).

RNA from fibroblasts infected in vitro with SSAV, GaLV-Br and GaLV-SEATO were analysed by Northern blotting with a complete viral probe. As shown in Fig. 2(a), lanes 1, 2 and 3, two RNA species of 8-4 kb and 2-9 kb were detected. The 8-4 kb mRNA hybridized to all of the cloned subgenomic probes, indicating that it contained the entire GaLV genome. The 2-9 kb mRNA hybridized to the LTR probe and the GAS-A (U3–R), GAS-B (env) and GAS-E probes (U5–gag), but not to GAS-C (pol) or GAS-D (gag–pol) probes, indicating that it is a spliced transcript which represents the env mRNA.
Fig. 2. Northern blot analysis of poly(A)^+ RNA isolated from cell lines infected in vitro and in vivo with primate retrovirus, hybridized to GalV-SEATO genomic and subgenomic viral probes, represented schematically in Fig. 1(b). (a) Lanes 1, 2 and 3, poly(A)^+ RNA isolated from fibroblasts infected in vitro with SAV, GalV-Br and GalV-SEATO respectively. Lanes 4 and 5, hybridization of RNA isolated from the original primary tumour lines 6G-1 and UCD-144 respectively, derived from the peripheral blood of GalV-H- and GalV-SF-infected leukaemic gibbons. (b) UCD-144 poly(A)-selected RNA hybridized to GalV-SEATO LTR probe (lane 1), to pGAS-1 subclone A (which corresponds to the U3 of the viral LTR) (lane 2), to subclone B (an env-specific probe) (lane 3), to subclone E (a gag and U5 probe) (lane 4), to subclone C (a probe containing pol sequences) (lane 5) and to subclone D (a probe containing mostly 3’ gag sequences) (lane 6). The sizes (kb) of viral mRNA are indicated.

RNA from the 6G-1 and UCD-144 cell lines was also analysed by Northern blotting (Fig. 2a, lanes 4 and 5). These are lymphoid leukaemic cell lines established from the peripheral blood of gibbon apes with lymphoid leukaemia and infected with two different members of the GalV family of viruses. The 6G-1 cells (infected with GalV-H) have some phenotypic properties of early or immature T-cells, including the presence of terminal transferase (Sarin et al., 1980), while the UCD-144 cells (infected with GalV-SF) resemble more mature, immunocompetent T-cells, in that they produce T-cell growth factor and lack terminal transferase (Rabin et al., 1981).

The pattern of viral mRNA present in 6G-1 cells was similar to that observed with fibroblasts infected with GalV-Br, GalV-SEATO or SAV. As shown in Fig. 2(a), lane 4, viral mRNA bands of 8.4 and 2.9 kb were detected. The 2.9 kb band hybridized to the subgenomic probes in a pattern consistent with that expected of a spliced env mRNA. The 8.4 kb mRNA hybridized with all the probes. No other bands of viral RNA were evident.

In contrast, RNA from the UCD-144 cell line (Fig. 2a, lane 5) gave three prominent bands on Northern blots, two of which (8.4 kb and 2.9 kb) appeared to be identical with the mRNAs in the other cell lines by analysis with subgenomic probes. In addition, an intense band of viral mRNA of 6.4 kb was evident. This RNA hybridized to the LTR, GAS-A (U3-R), GAS-B (env) and GAS-E (U5-gag) probes, but not to GAS-C (pol) or GAS-D (gag-pol), indicating that it contains LTR and some gag and env sequences but appears entirely to lack pol sequences (Fig. 2b). The GAS-C and GAS-D probes together comprise 3.0 kb of the viral genome. This means that viral sequences can account at most for approximately 5.4 kb of the 6.4 kb transcript, and that at least 1.0 kb may be non-viral. This is represented schematically in Fig. 1(b).
Southern blots were performed using DNA from UCD-144 and 6G-1. In both instances a pattern of viral sequences was observed which is most consistent with an oligoclonal proviral population. Using restriction enzymes that cut in the LTR of GaLV-H (SstI, KpnI and PstI), only complete copies of provirus were detectable in the 6G-1 cell line. In contrast, several variant forms of GaLV were evident in both chromosomal and low molecular weight Hirt DNA preparations from the UCD-144 cell line. One form (Form A) generates restriction enzyme fragments which correspond to the GaLV-SF provirus cloned and partially sequenced by Scott et al. (1981) (Fig. 1a). An EcoRI restriction fragment of 3.15 kb was derived from this form of the GaLV-SF provirus and, as expected, hybridized to the complete genomic GaLV-SEATO probe (Fig. 3, lane 1) and to GAS-C (lane 2) and GAS-D (not shown) but not to GAS-B (lane 3), GAS-E (not shown) or GAS-A (lane 4). Two other DNA fragments of 6.1 kb and 4.3 kb hybridized to the complete GaLV-SEATO probe (lane 1) as well as GAS-B (lane 3) but not to GAS-C (lane 2) or GAS-A (lane 4). The 6.1 kb but not the 4.3 kb fragment hybridized to GAS-E (not shown). The hybridization pattern observed with the 6.1 kb fragment (positive with gag and env but negative for pol) is consistent with the presence of a defective virus from which the 6-4 kb transcript observed in UCD-144 cells could conceivably have originated (schematically shown in Fig. 1b). The 4.3 kb EcoRI DNA fragment most likely represents another defective provirus also present in this cell line.

In this report, we have characterized viral DNA and viral RNA transcripts from two primary tumour T-lymphoid cell lines, 6G-1 and UCD-144, both of which were established from the peripheral blood of gibbon apes with lymphocytic leukaemias. The cell lines differ, however, in that the UCD-144 cells have a phenotype suggestive of a more differentiated T-cell and produce GaLV-SF, whereas 6G-1 cells resemble early or immature T-cells and produce a different strain of GaLV (GaLV-H). Analyses of these two lines by Southern and Northern blotting have
revealed several interesting features. First, 6G-1 cells do not contain a detectable defective virus. Second, the 6G-1 cells do not produce any unusual viral transcripts. Since these cells thus do not apparently contain a defective transforming virus or utilize the GaLV-H promoter for transcription of downstream cellular genes, it is possible that the leukemogenic event(s) related to virus infection occurred transiently and early in the disease. It is equally possible, however, that enhancer sequences in the GaLV-H LTR induce transcription of sequences which are not covalently linked to a viral leader sequence. A third finding, and one in contrast to the findings in 6G-1 cells, is that UCD-144 cells produce a viral mRNA species of 6.4 kb in addition to the gag-pol and env mRNAs. This transcript appears to encode at most 5.4 kb of GaLV sequences, and consequently could contain at least 1.0 kb of non-GaLV sequences. The viral sequences include LTR, gag and env but not pol or the 3' gag sequences, indicating that the 6.4 kb transcript contains both 3' and 5' viral sequences, and that the middle of the viral genome is absent and apparently replaced by non-GaLV sequences. Finally, UCD-144 cells contain several defective proviruses, one of which could account for the presence of the 6.4 kb viral mRNA from a defective transforming virus (the pol gene and possibly part of the gag and env gene are apparently replaced by non-GaLV sequences), and it is tempting to speculate that the 6.4 kb transcript derives from such a virus in this cell line. The presence of a defective transforming virus in the UCD-144 cell line has not been reported, but this may be because a suitable target cell has not been identified. Molecular cloning should provide the means for a better characterization of this defective recombinant provirus in the UCD-144 cell line.

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REFERENCES


Short communication


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