Human Adult T-Cell Leukaemia Virus Is Distinct from a Similar Isolate of Japanese Monkeys

By NAOKI YAMAMOTO,1 MORIHISA OKADA,1 YORIO HINUMA,1 FRIEDRICH W. HIRSCH,2 TORU CHOSA,3 JOSEF SCHNEIDER3 and GERHARD HUNSMANN3.

1 Institute of Virus Research, Kyoto University, Kyoto 606, Japan, 2 Medizinische Klinik Universität Freiburg, Federal Republic of Germany and 3 Deutsches Primatenzentrum, Abteilung Virologie und Immunologie, Kellnerweg 4, D-3400 Göttingen, Federal Republic of Germany

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SUMMARY

We have compared the structural polypeptides of an adult T-cell leukaemia (ATL) virus (ATLV) isolate from a Japanese patient with ATL with those of a similar virus derived from a Japanese macaque monkey. Both are distinct but related entities. Their core polypeptides p19 could not be distinguished, but p24, another core polypeptide, and their envelope glycopolypeptides differ. The human virus directs the synthesis of a single intracellular glycopolypeptide, gp68, while the macaque virus specifies two such glycopolypeptides, gp57 and gp50. Furthermore, the glycopolypeptides of both viruses are serologically distinct. Thus, these viruses represent subtypes of the ATLV family and the macaque virus is apparently not involved in human ATL.

Human adult T-cell leukaemia (ATL) was first described in Japan (Takatsuki et al., 1977; Uchiyama et al., 1977; T- and B-cell Malignancy Study Group, 1981). Later, a virus, HTLV or ATLV,† related to ATL was isolated (Poiesz et al., 1980, 1981; Hinuma et al., 1981; Yoshida et al., 1982; Robert-Guroff et al., 1982; Kalyanaraman et al., 1983; Shimoyama et al., 1982). Subsequently Japanese monkeys (Macaca fascicularis) were found to be naturally infected with a similar agent (Miyoshi et al., 1982; Hayami et al., 1984). Since ATLV appears to be involved aetiologically in ATL endemic in southwestern Japan (Takatsuki et al., 1977; Uchiyama et al., 1977; T- and B-cell Malignancy Study Group, 1981) it is important to compare a monkey isolate with a prototype human ATLV.

A unique cell line designated JM6 (M. Okada, unpublished) has been established by co-cultivation of human cord blood lymphocytes with peripheral lymphocytes from a Japanese macaque positive (immunofluorescence titre 1:4) for antibodies to ATL-associated antigens (ATLA) (Hinuma et al., 1981). Chromosome analysis revealed that the cells were of monkey origin. JM6 cells lack both T- and B-cell markers but over 70% of them express ATLA. JM6 and human MT-2 cells (Miyoshi et al., 1981) producing MT-2 ATLV were cultured in RPMI 1640 medium supplemented with 20 or 10% foetal bovine serum, respectively.

† We prefer to discriminate ATLV (adult T-cell leukaemia virus) from HTLV (human T-cell leukaemia virus) for the following reasons. (i) HTLV was isolated first in the U.S.A. from a black patient with mycosis fungoides (Poiesz et al., 1980). The prototype cell line producing HTLV is HUT 102. ATLV was isolated from a Japanese patient with ATL (Yoshida et al., 1982). The prototype cell line producing ATLV is MT-2. These cell lines have distinct biological and biochemical characteristics, which could be explained by distinct biological activities of HTLV and ATLV. Later it was found that another virus isolated from a Japanese ATL patient is biochemically very similar to the HTLV isolate (Watanabe et al., 1984) but minor differences in their sequence have been detected (Copeland et al., 1983). Thus both viruses represent independent isolates of the same viral subtype. (ii) The only disease the virus is regularly and specifically associated with is ATL or its lymphoma variant (Hinuma et al., 1981; Yoshida et al., 1984). Therefore these viruses are more appropriately named ATLV. (iii) Similar viruses were isolated from numerous Old World monkey species. These cannot be addressed as HTLV. Naming other human isolates obviously not associated with human leukaemias HTLV II and III appears likewise inappropriate. (iv) Until a nomenclature is agreed by an official international committee, we will discriminate both isolates and favour the more precise name ATLV for viruses associated with ATL.
Fig. 1. (a) Immunoprecipitation of polypeptides specified by Japanese monkey JM6 ATLV. Human ATLV-producing MT-2 cells and monkey ATLV-producing JM6 cells were labelled with radioactive precursors as indicated. Immunoprecipitates of cell extracts were analysed by PAGE. Briefly, 10 ml of a cell suspension (8 x 10⁶ per ml) were labelled for 10 h with 50 μCi/ml [³⁵S]cysteine or [³H]glucosamine (Amersham) in medium without cysteine or glucose and supplemented with dialysed foetal bovine serum. After solubilization of the cells with low salt extraction buffer (LSEB) the cell extract was reacted with human or rabbit serum positive or negative for ATLV antibodies. Immune complexes were harvested with Protein A-bound Sepharose (Pharmacia), washed and separated on a 9 to 16% gradient gel under reducing conditions. Gels were treated for fluorography. For immunoprecipitation, we used anti-ATLV antibody-negative human (lane 1), anti-ATLV antibody-positive serum from an ATL patient (lane 2), rabbit negative control serum (lane 3), rabbit anti-gp68 serum (lane 4), rabbit anti-p24 serum (lane 5) and rabbit anti-p19 serum (lane 6). [³⁵C]-labelled marker proteins (M) were run on the same gel (bovine serum albumin, 69000; ovalbumin, 45000; chymotrypsinogen, 25000; cytochrome c, 12500). The nine lanes on the right were run on the same gel as those on the left. (b) Western blots of MT-2 and JM6 cells. Cytosol extract was separated by two-dimensional gel electrophoresis. The unstained protein patterns were transferred to nitrocellulose sheets by the Western blot technique.
Both cell lines were labelled with \([^{35}S]\)cysteine or \([^{3}H]\)glucosamine (Yamamoto et al., 1982; Schneider et al., 1984a) for 10 h. Cytoplasmic extracts of labelled cells were incubated with antisera from ATL patients or rabbits immunized with isolated MT-2 ATLV p19, p24 or gp68 (Schneider et al., 1984b). Antigen–antibody complexes were adsorbed to Protein A–Sepharose and analysed by electrophoresis on polyacrylamide gels (PAGE) (Schneider et al., 1984a). From cysteine-labelled JM6 cells, six polypeptides were precipitated with an anti-ATLA-positive serum from an ATL patient (Fig. 1a). These polypeptides were designated according to their apparent molecular weights as p57, p50, p43, p36, p24 and p19. From MT-2 cells the same serum precipitated several ATLA-specific polypeptides (Yamamoto et al., 1982; Yamamoto & Hinuma, 1982; Schneider et al., 1984a) such as gp68, p53, p43, p36, p28, p19 and p15. Rabbit antisera against MT-2 ATLV p19 and p24 precipitated the respective polypeptides equally well from both monkey and human cells. In addition, a putative precursor of MT-2 ATLV core polypeptides (Schneider et al., 1984b) of about 50000 mol. wt. was precipitated with the p19 and p24 antisera from JM6 and MT-2 cells. In contrast, rabbit anti-gp68 serum precipitated its respective antigen only from MT-2 but not from JM6 cells. However, the latter cells did contain virus-specific glycoproteins. When JM6 cells were labelled with glucosamine two polypeptides corresponding to the cysteine-labelled p61 and p52 became precipitable with the human antiserum. But again these glucosamine-labelled polypeptides, gp57 and gp50, were not precipitated with the anti-gp68 rabbit serum.

Radioimmunoprecipitation assays have been devised for the titration of antibodies to MT-2 ATLV core polypeptides p19 and p24 as well as to gp68, which is the major precursor (Schneider et al., 1984a) to the env glycoprotein gp46 (Yamamoto et al., 1982; Schneider et al., 1984a). \(^{125}\)I-labelled purified MT-2 ATLV polypeptides were precipitated by serial dilutions of sera from ATL patients (Yamamoto et al., 1983a), non-human primates (Hunsmann et al., 1983) and rabbits immunized with purified viral components (Schneider et al., 1984b). These assays were adapted to measure by competition the concentration of cross-reacting antigens in crude mixtures. As shown in Fig. 2(a to c), viruses and extracts from both JM6 and MT-2 cells competed in the p24 assay with slopes and endpoints similar to either homologous human or rabbit antibodies or heterologous macaque antibody. Thus, p24 of JM6 and MT-2 cells were indistinguishable in this test. However, significant differences were observed between both viruses with the gp68 competition assay (Fig. 2d, e). MT-2 cell extracts competed with both rabbit and human antibodies while JM6 extracts did not compete with the rabbit antibodies. Extracts of Raji cells were inactive in these tests. To characterize the polypeptides specified by the human and monkey isolates further we performed Western blotting (Towbin et al., 1979) with a rabbit anti-MT-2 ATLV p24 serum (Schneider et al., 1984b) after two-dimensional electrophoresis (O'Farrell, 1975) of MT-2 and JM6 cell extracts. Details of this technique are described elsewhere (Hirsch et al., 1983). The results show that p24 of both MT-2 ATLV and JM-6 ATLV have similar molecular weights but distinct isoelectric points of 6-4 and 7-0, respectively (Fig. 1b). Similar experiments with a p19 serum (Schneider et al., 1984b) did not reveal differences (not shown).

Taken together, our tests could not discriminate p19 core polypeptides of human MT-2 ATLV and JM6 ATLV. However, these viruses apparently differ in their p24 core polypeptide and in their envelope glycopolypeptides. JM6 ATLV specifies two glycopolypeptides, gp57 and gp50, while the predominant intracellular glycopolyopeptide of cells infected with human MT-2 ATLV is gp68. Moreover, the rabbit antiserum raised against purified gp68 from MT-2 cells did not react with the two glycopolypeptides precipitated from monkey virus-infected cells with ATL patient sera. Whether these two glycopolypeptides originate from distinct viral genomes remains to be determined. However, JM6 ATLV apparently is a subtype distinct from the ATL-associated human MT-2 ATLV. Interestingly, Hayami et al. (1984) discovered that, in Japanese
monkeys, serum antibodies to human ATLV are not restricted to animals living in ATL endemic areas. Apparently Japanese monkeys are not the reservoir for ATLV infection of humans and vice versa. Preliminary data suggest that an ATLV isolated from African green monkeys (Cercopithecus aethiops) (Yamamoto et al., 1983b) is another distinct subtype and baboons may be infected with another ATLV-like virus (Guo et al., 1984).

Even though the macaque ATLV is probably not involved in ATL, elucidation of its biological activities may help to understand better the role of human ATLV subtypes in ATL. Moreover, a detailed comparison of virus-specific glycopolypeptides of HTLV/ATLV detected in man (Yamamoto et al., 1982; Hunsmann et al., 1983; Yamamoto et al., 1983a; Kalyanaraman et al., 1983; Fleming et al., 1983) and monkeys (Yamamoto et al., 1983b; Hunsmann et al., 1983; Ishida et al., 1983; Miyoshi et al., 1983; Hayami et al., 1984; Yamamoto et al., 1984; Guo et al., 1984) will allow further discrimination between subtypes and may shed light on the evolution of this interesting group of viruses.

Fig. 2. (a to c) Competition radioimmunoassays (RIA) for MT-2 ATLV p24. The RIA was performed with 125I-labelled p24 and a limiting dilution of a hyperimmune rabbit antibody to MT-2 ATLV p24 (a), serum from an ATL patient (b) or a seropositive Japanese monkey (c). Serial dilutions (10 µl) of the solubilized cells (10^6 per ml) or MT-2 ATLV in a reaction buffer (1:1 mixture of LSEB and PBS pH 7.2, containing 0.2 mg/ml ovalbumin and 1% anti-ATLV-negative human serum) were incubated with 1.5 µl of the appropriate serum dilution for 1 h at 37 °C. Labelled MT-2 ATLV p24 (20000 to 100000 c.p.m. in 40 µl reaction buffer) was added and the mixture incubated overnight at 4 °C. Two mg of Protein A-Sepharose was added per tube. After 30 min at 4 °C, the Sepharose was washed three times with high salt extraction buffer and the residual radioactivity in each tube was determined. For competition, extracts were used of JM6 cells (△), JM6 ATLV (△), MT-2 cells (●), MT-2 ATLV (○) and Raji cells (X). (d, e) RIA for MT-2 ATLV gp68. The purification of gp68 has been reported elsewhere (Schneider et al., 1984a, b; Yamamoto et al., 1983a). The RIA was performed with 125I-labelled MT-2 ATLV gp68 and a limiting dilution of a serum from an ATL patient (d) or hyperimmune rabbit antiserum to MT-2 ATLV gp68 (e).
REFERENCES


Short communication


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