The trans-activating C-type retroviruses share a distinct epitope(s) that induces antibodies in certain infected hosts

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Using sera from hosts infected with bovine leukaemia virus (BLV), human T cell lymphoma virus types I and II (HTLV-I and -II), or simian T cell lymphoma virus type I (STLV-I), we found that the major gag proteins of these viruses cross-react immunologically. The specificity of this cross-reactivity was demonstrated by absorption using purified viral proteins, virus lysates and extracts of infected cells. The data strongly suggested that the cross-reacting epitope(s), referred to as CE, differs from those responsible for cross-reactions between the major gag proteins of HTLV-I, HTLV-II and STLV-I, and between those of BLV and HTLV-I reported previously. The prevalence of antibodies to CE was low, even amongst infected hosts with high titres to other epitopes present in the major gag proteins of the homologous viruses. CE was not detected in any of the other C- or D-type retroviruses, or lentiviruses examined. Therefore, it is likely that CE can be used to define serologically a subgroup of C-type retroviruses, the genomes of which display unique features and functional activities.

Introduction

Initial characterization studies showed that the bovine leukaemia virus (BLV) differs from all previously identified C-type leukaemia retroviruses in several important properties, including a smaller major core protein lacking the inter- (gs3) and intra species (gs1)-specific antigenic determinants (Ferrer, 1972; Gilden et al., 1975; McDonald & Ferrer, 1976; McDonald et al., 1976), a reverse transcriptase preferring magnesium to manganese (Gilden et al., 1975; Graves et al., 1977) and the ability to induce syncytium formation (Diglio & Ferrer, 1976). Based upon these differences it was suggested (Ferrer, 1972; Diglio & Ferrer, 1976; McDonald & Ferrer, 1976) that BLV may be the prototype of a new family or subgroup of C-type retroviruses.

This hypothesis was substantiated when human T cell lymphoma virus type I (HTLV-I), the first human C-type leukaemia retrovirus, was identified and found to share distinctive properties with BLV (for a review see Weiss, 1984). Subsequently, it was found that the genomes of BLV and HTLV-I contain a coding region, X, which is responsible for the trans-acting transcriptional activity of these viruses. A similar region has been identified in the genomes of HTLV-II and simian T cell lymphoma virus type I (STLV-I). The trans-activating proteins encoded by the X region most likely play an important role in the expression, replication and pathogenicity of these viruses (for reviews see Yoshida, 1987; Schupbach, 1989; Nerukar et al., 1990).

Using sera from certain infected hosts we showed that BLV, HTLV-I, HTLV-II and STLV-I share an antigenic determinant(s) which distinguishes them from other retroviruses. The presence of antibodies against this determinant is restricted even in infected hosts having high titres of antibodies against the major gag proteins of homologous viruses.

Methods

Sera and serum IgG. Sera were collected from cows, naturally infected with BLV, which had histologically confirmed lymphosarcoma or were healthy carriers. Some of the latter had persistent lymphocytosis, a frequent benign response of cattle to BLV infection (Ferrer et al., 1979). All the sera were positive in a radioimmunoassay (RIA) using 125I-labelled BLV p25 (RIA-BLV p25) (McDonald & Ferrer, 1976) and/or an indirect immunofluorescence test on target BLV-infected NBC-13 cells (Ferrer et al., 1974). Control sera were from cattle free of BLV infection which were repeatedly negative for BLV antibodies, as determined by RIA using either p25 (McDonald & Ferrer, 1976) or the virion envelope glycoprotein (gp51) as antigen (Gupta & Ferrer, 1981).

Sera from sheep experimentally infected with BLV and from BLV-free sheep produced previously (Kenyon et al., 1981). Sera were obtained also from rabbits and chimpanzees which had been inoculated with blood leukocytes from BLV-infected cows and were continuously positive for BLV antibodies for a 4 to 5 year period, as determined by RIA-BLV p25. The chimpanzees were maintained at the Yerkes Regional Primate Center, Emory University, Atlanta, Ga., U.S.A., where they were inoculated and monitored by Dr Harold 0001-0272 © 1991 SGM
McClure. The bovine peripheral blood leukocytes (PBLs) used for the inoculation of these animals were supplied by our laboratory.

Human sera positive for antibodies to HTLV-I were kindly provided by Drs W. Hardy and E. Zukerman (Memorial Sloan Kettering Cancer Institute, N.Y., U.S.A.), Drs M. McGrath and V. Ng (San Francisco General Hospital, Ca., U.S.A.), Dr S. Foung (Stanford University Medical Center, Ca., U.S.A.), Dr M. H. Kaplan (North Shore University Hospital Cornell Medical College, Manhasset, N.Y., U.S.A.) and Dr S. Alexander (Biotech Research Laboratories Inc., Rockville, Md., U.S.A.). Human anti-HTLV-I sera were also purchased from the New York Blood Center and from Serologicals. The pattern of Western blot (WB) reactivity of all these sera was typical of HTLV-I. Drs Foung, Kaplan and Alexander also supplied sera from people infected with HTLV-II. Additional human anti-HTLV-II sera were a gift from Dr H. Lee (Abbott Laboratories, North Chicago, Ill., U.S.A.) or were purchased from Serologicals. HTLV-II infection was demonstrated in donors either by the polymerase chain reaction or Southern blot analysis of DNA from PBLs. All the HTLV-II sera reared in WB with p24 and at least one other HTLV-II protein; these sera also reacted with HTLV-I p24, but not with p19 or other proteins from this virus.

Anti-STLV-I-positive sera from mangabeys monkeys were a gift from Dr H. McClure. Dr. A. Voevodin (USSR Academy of Medical Sciences, Sukhumi, U.S.S.R.) kindly supplied a pool of two baboon sera which were anti-HTLV-I-positive by WB and other serological tests (Voevodin et al., 1985).

IgG from bovine serum Se-678 was purified using the GammaBind G-Agarose Kit from Genex Corporation.

**Viral proteins.** Sucrose gradient-purified BLV was disrupted by sonication and NP40 (0.5% in 20 mM-Tris-HCl pH 7.5, 100 mM-NaCl, 1 mM-EDTA). The preparation was then centrifuged (12000 g, 15 min) and applied to a DuPont GF-250 HPLC column. The eluted proteins were monitored with an LKB diode array detector and further characterized by SDS-PAGE. The fractions containing p25 were passed through a Mono Q column (Pharmacia); BLV p25 in the flowthrough fraction had a purity greater than 95% iodination of purified BLV p25 was done by the chloramine T method (Greenwood et al., 1963).

Recombinant HTLV p24 and recombinant human immunodeficiency virus (HIV) p24 were kindly supplied by Drs J. Seals (Cambridge BiScience, Worcester, Mass., U.S.A.) and S. Petteyew (SmithKline and French Laboratories, King of Prussia, Pa., U.S.A.), respectively.

**Viral preparation and cell lysates.** BLV was purified by density gradient centrifugation. Density gradient-purified preparations of HTLV-I and -II were purchased from Hillcrest; preparations of density gradient-purified simian sarcoma virus (SSV), Mason-Pfizer monkey virus (MPMV), gibbon ape leukemia virus (GALV), Moloney sarcoma virus B (MSV), murine leukemia virus (AKR-MLV) and Rous sarcoma virus (Harris strain) (RSV) were purchased from the Research Resources Branch, National Cancer Institute (Bethesda, Md., U.S.A.). Density gradient-purified feline leukemia virus (FeLV), disrupted by Tween and ether treatment, was a gift from Dr F. Noronha (Cornell University, Ithaca, N.Y., U.S.A.). The equine infectious anemia virus (EIAV) was purified by density gradient purification from the supernatant of a chronically infected culture of foetal monkey dermal cells kindly supplied by Dr R. Montefioro (Louisiana State University, Baton Rouge, La., U.S.A.) (Rasty et al., 1990).

Cell extracts were prepared as follows. After washing three times, cells were resuspended in buffer A (20 mM-Tris-HCl pH 7.5, 20 mM-NaCl and 1 mM-EDTA) and lysed by adding an equal volume of buffer B (20 mM-Tris-HCl, 180 mM-NaCl, 1 mM-EDTA, 0.1% NP40, 0.2% sodium deoxycholate). The extracts were then frozen and thawed three times, and clarified by centrifugation (12000 g, 2 min); extracts contained the equivalent of 5 × 10^7 cell/ml.

The HTLV-I-infected MT-2 (Migushi et al., 1981) and the non-infected H9 (Popovic et al., 1984) T cell lines were obtained from Dr P. Sarin (National Cancer Institute, Bethesda, Md., U.S.A.). The HTLV-II-infected cell line 729 p6 neo (Chen et al., 1984) was a gift from Dr L. Chen (University of California, Los Angeles, Ca., U.S.A.); Dr R. Desrosiers (New England Regional Primate Research Center, Southborough, Mass., U.S.A.) kindly supplied cell line STLVL-I-39-83 (Daniel et al., 1988).

**Serological tests.** RIA-BLV p25 was performed by the double antibody method (McDonald & Ferrer, 1976); WBs were performed essentially as described by Johnson et al. (1984). A preparation of density gradient-purified virus or cell lysate was electrophoresed on a 10% polyacrylamide gel (Laemmli, 1970), and electrophoretically transferred to Immunobilon membranes (Millipore). The membranes were treated with MTBS (5% non-fat dried milk, 50 mM-Tris-HCl pH 7.4, 200 mM-NaCl, 0.1% Antifoam-A emulsion) to block additional non-specific protein-binding sites, and then exposed to the test sera at 1:50 and 1:100 dilutions in MTBS. Binding of the sera was detected using alkaline phosphatase-conjugated, affinity-purified antibodies against the IgG of the donor species. Anti-bovine, anti-sheep, anti-rabbit and anti-human IgG conjugates were purchased from Jackson Immunoresearch Laboratories.

The competitive RIA was performed as described (McDonald & Ferrer, 1976). Competing antigen preparations were diluted in RIA buffer containing 10 mg/ml bovine serum albumin and 10 μl was added immediately before the serum.

For WB absorption experiments, 10 μl serum was diluted 1:20 and mixed with the cell extract, virus lysate or purified virion protein, incubated for 2 h at room temperature and overnight at 4 °C, and tested at 1:50 or 1:100 dilutions in MTBS.

**Results**

**Reactivity of sera from BLV-infected hosts with human and simian C-type retroviruses**

Lysates of density gradient-purified viruses were tested by WB with sera collected from 62 BLV-infected cows with lymphosarcoma (RIA-BLV p25-positive). All sera reacted with the p25 and gp51 antigens of BLV; seven sera reacted with HTLV-I p24 and a few others occasionally showed very faint p24 reactivity when tested at a dilution of ≤ 1:50. Fig. 1 shows the results of a representative WB experiment with bovine sera against BLV and HTLV-I. The serum in lane 2 showed the strongest reactivity with HTLV-I p24; purified IgG from this serum showed the same reactivity (lane 1). Lanes 5 to 9 correspond to some of the many sera that reacted strongly with BLV p25, but failed to recognize HTLV-I p24. HTLV-I p24 was not recognized by any of the 20 sera from asymptomatic BLV carriers tested, although they did react with BLV p25 by both WB and RIA (data not shown).

The bovine anti-BLV sera that recognized HTLV-I p24 also reacted with HTLV-II p24. Similarly, HTLV-II p24 was not recognized by any of the bovine anti-BLV
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Fig. 1. WB reactivity of bovine sera with lysates of BLV (a) and HTLV-I (b). Lanes 1, IgG purified from the serum in lane 2; lanes 2 to 9, anti-BLV sera, lanes 10 and 11, BLV-negative sera; lanes 12, anti-bovine IgG conjugate control only; lanes 13, human anti-HTLV-I-positive serum; lanes 14, human anti-HTLV-I-negative serum; lanes 15, anti-human IgG conjugate control only. Mf markers are shown (lanes M).

Of 23 sera from BLV-infected sheep, six which reacted strongly with p25 and other BLV antigens by both WB and RIA formed a prominent band with HTLV-I p24; seven other sheep anti-BLV sera reacted weakly with HTLV-I p24. All the sheep sera formed prominent bands with p25 and other BLV proteins. The ability of sheep anti-BLV sera to cross-react with HTLV-I p24 did not necessarily correlate with the strength of reaction with BLV p25. The WB reactivity of the sheep sera with HTLV-II was identical to that with HTLV-I (data not shown).

As shown in Fig. 2, preincubation with native, highly purified BLV p25 completely removed the reactivity of sheep anti-BLV serum Se-349 with both BLV p25 and HTLV-I p24, but did not affect its reactivity with other BLV proteins; the same results were obtained with bovine anti-BLV serum Se-678 (data not shown). These results demonstrate the specificity of the cross-reactions observed.

The bovine and sheep sera showing the strongest reactivity against HTLV-I and -II p24 also reacted with p24 when tested against an extract of STLV-I-infected cells. This band was not seen when the STLV-I cell extract was tested with bovine and sheep sera that reacted strongly with BLV p25 but did not recognize HTLV-I and -II p24 (Fig. 3).

Sera from five chimpanzees and nine rabbits experimentally infected with BLV reacted strongly with BLV p25 by RIA and WB, but showed no reactivity with HTLV-I p24 (data not shown).

Reactivity of HTLV-I-, HTLV-II- or STLV-I-positive sera with BLV

Lysates of BLV were tested by WB with 55 human sera which showed a pattern of WB reactivity typical of
HTLV-I. Of the 55 sera tested, two formed a distinct band with BLV p25 and six reacted very weakly (Fig. 4). The sera in lanes 1 and 2 did not differ appreciably in the strength with which they recognized the HTLV-I proteins, but differed markedly in their reactivity with BLV p25. The serum in lane 1 occasionally formed a p12 band with BLV; the nature of this band was not investigated.

A weak, but distinct, reactivity with BLV p25 was seen with one of 12 simian anti-STLV-I sera tested; all these sera reacted strongly in WB with the p24 and p19 proteins of HTLV-I, and occasionally with other proteins (data not shown).

Of 41 human anti-HTLV-II sera tested, four reacted with BLV p25, but did so only weakly. These sera all bound to p24 and at least one other HTLV-II protein. They also reacted with HTLV-I p24, but not with p19 or other polypeptides (data not shown).

The human anti-HTLV-I serum Se-739 (Fig. 4, lane 1) which showed the strongest reactivity with BLV p25 by WB also immunoprecipitated this protein in RIA (Fig. 5). BLV p25 was also precipitated by the serum of a BLV-infected chimpanzee; no precipitation was seen with human serum Se-759 which by WB reacted strongly with HTLV-I. The specificity of the immunoprecipitation of BLV p25 by human anti-HTLV-I serum Se-739 was demonstrated by the fact that competition was seen with recombinant HTLV-I p24 and BLV p25, but not with recombinant HIV p24 (Fig. 6). As determined by WB, extracts of cells infected with STLV-I, HTLV-I, HTLV-II or BLV, but not uninfected H9 cells, completely inhibited the binding of human anti-HTLV-I serum Se-
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Fig. 5. Human anti-HTLV-I and chimpanzee anti-BLV sera immuno-precipitate $^{125}$I-labelled BLV p25. ○, human anti-HTLV-I serum (Se-739); ×, human anti-HTLV-I serum (Se-759); △, human anti-HTLV-I-negative serum; □, serum from a BLV-infected chimpanzee.

Fig. 6. Purified HTLV-I p24 and BLV p25, but not HIV p24, compete with the precipitation of $^{125}$I-labelled BLV p25 by human anti-HTLV-I serum Se-739. △, HTLV-I p24; ●, BLV p25; ■, HIV p24.

Fig. 7. Competition between serum Se-739 and various virus lysates for binding to BLV p25. Competing virus lysates: □, BLV; ●, HTLV-I; △, HTLV-II; ×, FeLV; ■, GALV; ○, MSV; ▲, MLV; ◯, RSV; +, MPMV; ○, EIAV; ●, SSV.

As shown in Fig. 7, although lysates of HTLV-I and -II competed effectively with the immunoprecipitation of $^{125}$I-labelled BLV p25 by human anti-HTLV-I serum Se-739, lysates of purified AKR-MLV, SSV, GALV, FeLV, MSV, RSV, EIAV and MPMV all failed to compete.

Antibody titres against BLV p25 of bovine and sheep sera showing differences in their reactivity with HTLV p24

As shown above, bovine and sheep anti-BLV sera which reacted with BLV p25 with comparable strength differed in their ability to recognize the major core proteins of HTLV-I, HTLV-II and STLV-I. To obtain a more definitive result we compared the antibody titres of these sera against BLV p25 by RIA. As shown in Fig. 8, the anti-BLV p25 titres of four sheep anti-BLV sera that did not recognize HTLV p24 were virtually the same as the titres of the sera which did react with this protein. The same results were obtained when several bovine anti-BLV sera were compared in terms of their WB reactivity with HTLV-I p24 and their antibody titre against BLV p25, as determined by RIA.
Discussion

Using sera from certain infected hosts, we have shown reciprocal cross-reactivity between the major core proteins of BLV, HTLV-I, HTLV-II and STLV-I. The specificity of the cross-reactivity between the BLV and HTLV-I proteins was confirmed by absorption experiments using BLV p25 purified to homogeneity and lysates of purified viruses. Sera from BLV-infected cattle and sheep which reacted with HTLV-I p24 also reacted with the corresponding proteins of HTLV-II and STLV-I; these proteins were not recognized by bovine and sheep anti-BLV sera that failed to react with HTLV-I p24. Moreover, lysates of purified HTLV-II and an extract of STLV-I-infected cells competed with the immunoprecipitation of 125I-labelled BLV p25 by a human anti-HTLV-I serum, as well as with the WB reactivity of a bovine anti-BLV serum with HTLV-I. Thus, HTLV-II and STLV-I p24 seem to share a common epitope(s) (CE) which is responsible for the cross-reactivity between the major core proteins of BLV and HTLV-I.

Several groups have shown that the p24s of HTLV-I, HTLV-II and STLV-I cross-react (for reviews see Schupbach, 1989; Nerukar et al., 1990). Consistent with these observations, all the human anti-HTLV-I sera tested react with the p24s of HTLV-II and STLV-I. Thus, the fact that only a few of the anti-HTLV-I sera react with BLV p25 suggests that CE differs from the epitope(s) responsible for the cross-reactivity between the p24s of HTLV-I, HTLV-II and STLV-I detected previously.

In previous studies (Kalyanaraman et al., 1981a, b; Robert-Guroff et al., 1982; Schupbach, 1989), anti-HTLV-I sera have failed to cross-react with the BLV major core protein in competitive RIA; however, hyperimmune goat anti-HTLV-I serum does react with BLV p25 after denaturation (Oroszlan et al., 1984). Similarly, applying a competitive ELISA, competition between a monoclonal antibody to BLV p25 and some human anti-HTLV-I sera has been demonstrated, but only after the antigen had been denatured (Onuma et al., 1987). These observations were interpreted as indicating that the cross-reactivities observed are due to sequential antigenic determinants present in the internal domain of the virion major core proteins which became exposed after denaturation. By contrast, denaturation of the virion protein does not seem to be required for the detection of CE; native BLV p25 is immunoprecipitated by a human anti-HTLV-I serum and eliminates the reactivity of bovine and sheep anti-BLV sera with HTLV-I. Likewise, lysates of HTLV-I or -II prepared under conditions unlikely to denature the virion gag proteins competed with the binding of BLV p25 with human anti-HTLV-I serum Se-739. From these observations it appears that CE differs from the epitope(s) responsible for the cross-reactivities between the major core proteins of BLV and HTLV-I reported previously by Oroszlan et al. (1984) and Onuma et al. (1987), and that, unlike this epitope, CE is present on the surface of the molecule.

Maruyama et al. (1989) have attempted to study the cross-reactivity between the BLV and HTLV-I gag proteins using sera from infected and vaccinated hosts. However, the data are difficult to evaluate, particularly because the identity of the bands in the WBs shown cannot be discerned accurately. Absorption experiments using viral proteins, virus lysates or cell extracts were not conducted and the specificity of the reactions was not established.

Oroszlan et al. (1982) have shown that the major core proteins of BLV and HTLV-I share significant homology in both the N- and C-terminal regions. It remains to be determined whether CE corresponds to any of these homologous sequences.

CE seems to be a weak immunogen because it is recognized by only some of the sera with strong reactivity against the major core proteins of homologous viruses. Elucidating the reasons for the restricted ability of the
infected hosts to recognize CE and determining the functional significance of the humoral response against it in terms of the clinical outcome of the infectious process may be important for understanding the biology and pathogenesis of BLV, HTLV-I, HTLV-II and STLV-I.

The detection of CE provides further evidence that BLV, HTLV-I, HTLV-II and STLV-I share a common likely that the presence of CE may be restricted to a transcriptional trans-activating activity. Thus, sera not detected in any of the other C-type retroviruses, nor detection and taxonomical classification of other, un-identified, C-type retroviruses of humans and animals.

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References


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