Isolation of Infectious Bovine Rhinotracheitis Virus from the Soft-Shelled Tick, *Ornithodoros coriaceus*

Abstract. *Infectious bovine rhinotracheitis virus was isolated from the soft-shelled tick (*Ornithodoros coriaceus*). Serological identification of the isolated viruses was confirmed by restriction endonuclease digestion of purified virus DNA. These isolations indicate that the soft-shelled tick may be a vector for infectious bovine rhinotracheitis virus. This may be the first reported isolation of a mammalian herpesvirus from an arthropod vector.*

Infectious bovine rhinotracheitis (IBR) virus (bovine herpesvirus type 1) is an important pathogen of cattle, causing infections that result in severe economic losses to the agricultural industry. Disease syndromes vary from subclinical respiratory disease to more acute disease and abortion (*1, 2*). Although IBR virus was first isolated in the western United States, it has a worldwide distribution (*3*). Mule deer (*Odocoileus lemionus*) are also susceptible to the virus (*4*). This is of particular interest since mule deer and domestic cattle sometimes occupy the same ranges in the western United States.

During studies of an unrelated disease of cattle, epizootic bovine abortion, we isolated viruses from ticks collected by means of CO_2 traps as described by Garcia (*5*). From soft-shelled ticks (*Ornithodoros coriaceus*) collected from deer bedding areas in the Sierra Nevada Mountains in 1979, we isolated a virus that produced cytopathic effects in Madin-Darby bovine kidney (MDBK) cells that were typical of IBR virus. The virus was typed serologically and specifically neutralized with antiserum to IBR obtained from D. McKercher (University of California, Davis). Ticks were again collected from the same areas in the Sierra Nevada in June and July of 1980 and 1981. The soft-shelled ticks were separated into groups of 15, washed to remove contaminating material in Eagle's minimum essential medium (Dulbecco's modification) supplemented with 10 percent fetal calf serum, and homogenized in a tissue homogenizer. The suspension was clarified by centrifugation at 2000*g* for 5 minutes. The supernatant fluids were then assayed for virus in MDBK cells.

To ensure that any virus isolated was not a result of viral contamination of the fetal calf serum or activation of latent IBR virus in the MDBK cells, uninoculated cells containing the same fetal calf serum were maintained as controls. In addition, pools of ticks were divided in half and subjected to virus isolation procedures at both the Agriculture Experiment Station and the Department of Microbiology, University of Nevada. These laboratories are physically separate and have different sources of fetal calf serum and cells. Virus was isolated from two separate collections obtained in June 1981 but not from the ticks collected during 1980. Virus from the ticks collected during 1981 produced cytopathic effects typical of IBR virus, and the suspension was assayed to determine the number of infectious virus particles. One suspension of 15 ticks contained 1×10^4 plaque-forming units of virus. The isolates could be neutralized with antiserum to IBR.

Although the isolated virus serologically resembled IBR virus, it remained to be determined whether it shared certain antigenic determinants with IBR virus or was in fact IBR virus. Herpes simplex virus types 1 and 2 are highly cross-reactive serologically; however, they produce clinically different diseases and differ significantly in their DNA homology (*6*).

The restriction enzyme pattern of the DNA of a virus is very useful for demonstrating genetic relations (*7*). To determine whether the restriction pattern of DNA from the virus isolates was similar to that of IBR virus, the various isolates and the Los Angeles strain of IBR virus (American Type Culture Collection) were plaque-purified and labeled by adding ^{32}P to IBR-infected MDBK cells (*7*). The virus was purified by velocity sedimentation in discontinuous gradients of 30 to 60 percent sucrose. Viral DNA was extracted by digestion with Pronase and was further purified by velocity sedimentation in continuous gradients of 10 to 30 percent sucrose (*8*). Figure 1 shows autoradiograms of Eco RI and Kpn I digests of the Los Angeles strain, the 1981 tick isolate, and the 1979 isolate. The DNA restriction patterns obtained with both enzymes are identical for the three isolates. In each isolate tested, the Eco RI digest resulted in the generation of seven fragments and the Kpn I digest resulted in the generation of ten fragments. The similarity between the isolates is somewhat surprising, since there is considerable minor variability in the restriction patterns of isolates of herpes simplex types 1 and 2. We have examined the restriction pattern for nine isolates of IBR virus; of these, six appeared identical and three exhibited some minor variations. Consequently, the restriction pattern between IBR isolates appears to be more stable than that between isolates of herpes simplex types 1 and 2. The identification was further confirmed by determining the density of the viral DNA in neutral CsCl gradients (1.73 g/cm^3) and its size in gradients of 10 to 30 percent neutral sucrose.

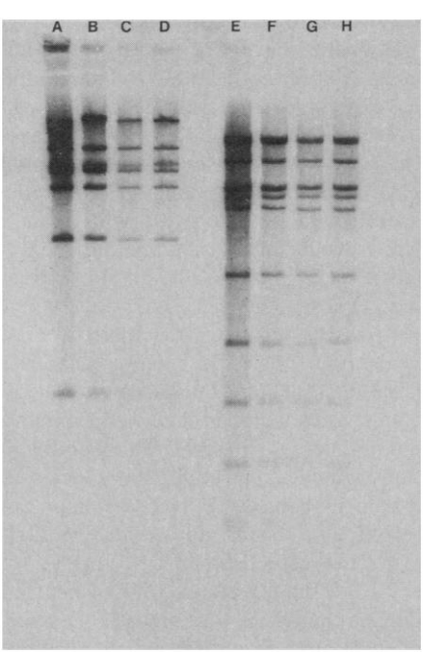

Fig. 1. Autoradiograph of Eco RI and Kpn I digestion products of DNA isolated from IBR virus (Los Angeles strain) and virus isolated from the soft-shelled tick (*Ornithodoros coriaceus*). Lanes *A* to *D* are Eco RI digests and lanes *E* to *H* are Kpn I digests of the 1981 tick isolate (*B, C, F,* and *G*), 1979 tick isolate (*D* and *H*), and the Los Angeles strain (*A* and *E*). Viral DNA was labeled by adding 1.0 mCi of ^{32}P (as H_3PO_4) to a culture of 10^7 cells in Dulbecco's phosphate-free media during viral DNA production. The supernatant was cleared by low-speed centrifugation and the virus was pelleted from the supernatant. Viral DNA was isolated by purifying virus particles in a discontinuous gradient of 30 to 60 percent sucrose. After pelleting the virus from the sucrose band, virus particles were digested with Pronase in the presence of sarcosyl and EDTA. The DNA was banded in a continuous gradient of 10 to 30 percent sucrose, and fractions were collected and dialyzed against 10 m*M* tris-HCl. DNA was precipitated in alcohol and brought to volume in 10 m*M* tris. Endonuclease digestions of DNA with Kpn I and Eco RI (Bethesda Research Laboratories) were carried out at 37°C for 6 hours (*7*). The digests were electrophoresed in 0.5 percent agarose gels for 15 hours at 100 mA and 40 V in tris-acetate, dried with a Bio-Rad gel dryer, and exposed with Kodak XAR-5 autoradiography film.

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The serological analysis and the restriction enzyme pattern of the tick isolates leave little doubt that the viruses were IBR virus. To our knowledge, this is the only reported example of a mammal-infecting herpesvirus isolated from an arthropod host. It is not known whether the presence of the virus in the ticks is a result of biological replication or mechanical transmission. The source of the virus that infected the ticks is also in question. We have found antibodies to IBR virus in both deer and cattle in the area of the Sierra Nevada (*9*) where the ticks were collected. Since the viremic stage of disease in an animal is relatively short, there must be very few animals in an area at a particular time capable of transmitting virus to ticks; consequently, it is puzzling that virus was isolated from three separate collections of ticks obtained over a 3-year period. The presence of IBR virus in each of these collections would be even more remarkable if the ticks were mechanically infected, since one would expect the virus to be present for only brief intervals. Hence it is important to determine (i) whether IBR virus can replicate in ticks, (ii) how long the virus remains infectious in ticks, (iii) whether ticks can be infected with IBR virus from feeding on viremic cattle and can then transmit the disease to normal cattle, (iv) whether IBR virus can be observed in the tick with the electron microscope, and (v) whether the virus can be mechanically transmitted without replication of the virus.

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Human Immunoglobulin Heavy Chain Genes Map to a Region of Translocations in Malignant B Lymphocytes

Abstract. *A human immunoglobulin heavy chain (γ4) gene is mapped by chromosome hybridization in situ. This gene is located at band 14q32, a site commonly involved in a chromosomal translocation characteristic of malignant B cells.*

Two types of genetic rearrangements are associated with certain malignant B (bone marrow derived) lymphocytes. One involves the rearrangements necessary to form active immunoglobulin genes (*1*). The other consists of less well understood chromosomal translocations observed in certain human B cell lymphomas and leukemias, translocations that join a part of chromosome 8 to one of the chromosomes (namely, 2, 14, or 22) that bear immunoglobulin genes (*2*). The association of these nonrandom translocations with the chromosomes that carry immunoglobulin determinants suggested that these recombinational events might be related (*3*). Klein (*4*) and Rowley (*2*) have postulated how these translocations might also induce or maintain the malignant state.

Initial experiments (*5*) with human hybrid cell lines had resulted in the assignment of the human genes coding for immunoglobulin heavy, κ, and λ chains to chromosomes 14, 2, and 22, respectively. Chromosomal hybridization techniques in situ (*6–8*) now allow us to map these genes to their chromosomal bands with greater precision. We have begun by mapping the human heavy chain constant region gene, γ4. We find that it is located on chromosome 14, band q32 (14q32), exactly the band to which a characteristic B cell lymphoma and leukemia translocation occurs.

The probe for these mapping studies consisted of a 6.6-kilobase (kb) cloned human DNA fragment that, by sequence analysis, has been shown to include the human γ4 constant region (Igγ4) gene and its associated flanking sequences. This fragment has been physically linked to genes corresponding to other γ subclasses and bears considerable homology to them (*9*). The fragment contains no reiterated sequences. It was subcloned in the plasmid pBR322 (Fig. 1), labeled with ^3H, and used as a probe of human mitotic chromosome spreads in an in situ hybridization reaction (*10*).

The appearance of a quinacrine-banded chromosome spread visualized with incident light fluorescence is shown in Fig. 2A. Under added visible light and when the plane of focus is raised to the level of the emulsion (Fig. 2B), a silver grain (arrow) is seen at the distal segment of the q (long) arm of one of the chromosomes 14 in this cell. The chromosome banding pattern stays clear for 1 to 2 minutes before fading. A burning out of the background fluorescence creates a circular halo around the chromosome spreads, thus making it easy to distinguish previously analyzed from unanalyzed spreads. Since the stain is fluorescent, the silver grains are clearly visualized against the green-white chromosomal background.

A histogram presenting data compiled from an analysis of 50 chromosomal spreads is shown in Fig. 3. The data represent the analysis of two observers working independently, one of whom was unaware of the identity of the hybridizing probe. The recordings by the two observers were essentially identical, and the experiment has been repeated with peripheral blood from three different sources. Between one and five grains were associated with chromosomes in each chromosome spread. The grain background was either in the same range or, in most of the spreads, lower than the number associated with chromosomes. Of the 50 cells represented in the histogram more than 60 percent contained at least one chromosome 14 with a silver grain at the distal end of the q arm. Approximately 30 percent of all the grains observed were located at 14q32.

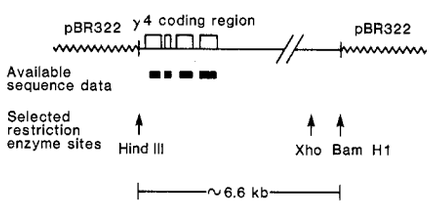

Fig. 1. Cloned human γ4 DNA fragment used as in situ probe. A human γ4 DNA segment was isolated from a partial Mbo I library (*21*) of human placental DNA fragments, packaged (*22*) in the λCH28 vector (*23*), and identified by in situ hybridization (*24*) with a previously cloned human γ4 DNA fragment that was initially identified through cross-hybridization to a cloned mouse γ chain cDNA probe (*25*). An approximate 6.6-kb Bam HI–Hind III fragment was subcloned into the plasmid pBR322. DNA sequence determination (*26*) in the first, second, and third domains and hinge region (filled boxes, data not shown) identified this subclone as γ4 genomic fragment when compared to known amino acid sequences of heavy chain constant regions (*27*).

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