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IDENTIFICATION OF AN INFECTIOUS LARYNGOTRACHEITIS VIRUS EQUIVALENT TO THE HERPES SIMPLEX VIRUS TYPE 2 MAJOR DNA BINDING PROTEIN (ICP8)

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Summary. — A region of homology between the genomic DNA of infectious laryngotracheitis virus (ILTV), an avian herpesvirus, and the region encoding the herpes simplex virus type 2 (HSV-2) major DNA binding protein (ICP8) has been detected by the use of nick translation and Southern blot analysis. Further, a monoclonal antibody directed against the HSV-2 ICP8 protein detected has antigenic cross-reaction with ILTV as demonstrated by indirect immunofluorescence.

Key words: *infectious laryngotracheitis virus*; *major DNA binding protein*; *herpes viruses*

Introduction

The avian herpesvirus, infectious laryngotracheitis virus (ILTV) is the causative agent of an economically important respiratory disease of chickens. The disease occurs worldwide with a sporadic occurrence of outbreaks which vary in virulence, ranging from very mild symptoms to severe disease and death. Recently, Leib *et al.* (1987) determined that the ILTV genome was approximately 160 kb in size (109×10^6 MW) and that the genomic structure is similar to that of pseudorabies virus, equine herpesvirus type 1 and infectious bovine rhinotracheitis virus, thus α-herpesviruses.

Apart from the studies of Kotiw *et al.*, (1984, 1986), Leib *et al.*, (1986, 1987) and York *et al.*, (1987) little work has been carried out on this virus at the molecular level. No direct comparison of ILTV and herpes simplex viruses (HSV), either at the DNA or protein levels has been reported. In this work we tested for cross-reaction between the HSV-2 major DNA binding protein (ICP8) and ILTV DNA and proteins. The HSV-2 ICP8 was chosen as it has previously been shown that many herpesviruses possess an equivalent protein that is conserved at either the DNA and/or amino acid level (Littler *et al.*, 1981; Yeo *et al.*, 1981; Kemble *et al.*, 1987; Maragos and May 1987; Buckmaster *et al.*, 1988). However, no such studies using DNA hybridization and

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monoclonal antibodies have been reported for a non-mammalian herpesvirus. Recently Buckmaster *et al.* (1988) reported sequence conservation at the amino acid level between the major DNA binding protein equivalents of the avian Marek's disease virus and herpesvirus of turkeys when compared with varicella-zoster virus and cytomegalovirus of humans.

It has been shown previously that the 4.5 kb *Bgl*II DNA fragment 0 contains at least a part, if not all, of the ICP8 coding region in HSV-2 (Buchman *et al.*, 1979; Sheppard *et al.*, 1985). This DNA fragment was used for hybridization with ILTV DNA. Also a monoclonal antibody directed against the HSV-2 ICP8 was tested by indirect immunofluorescence for cross-reaction with ILTV proteins.

Materials and Methods

Virus and cells. SA-2, the vaccine strain of ILTV used in Australia, was propagated and assayed in primary chicken kidney (CK) cells as described by Bagust (1986), except that Eagle's Basal Medium rather than medium 199 was used. Viral DNA was prepared using the Pignatti method (Pignatti *et al.*, 1979).

Restriction enzyme cleavage and Southern blot analysis. Restriction enzymes were purchased from Pharmacia, Australia and DNA digests were carried out under the conditions recommended by the manufacturer. Souther transfer was performed essentially as described by Southern (1975). DNA probes were labelled by nick translation as described by Rigby *et al.*, (1977). Hybridizations were carried out at 37°C in 10 × Denhardt s (0.2% Ficoll, 0.2% polyvinylpyrrolidone and 0.2% bovine serum albumin), 6 × SSC (0.9 M sodium chloride, 0.09 M sodium citrate), and 0.1% sodium dodecyl sulphate with no formamide and were washed twice at 68°C in 2 × SSC and 0.1% SDS.

Immunofluorescence. Subconfluent monolayeres of CK cells grown on glass coverslips were infected with ILTV. At 19 hr post-infection the cells were washed in phosphate buffered saliZe (PBS) and fixed for 10 min in acetone. The coverslips were reacted for 30 min with monoclonal antibody LP4 (McLean *et al.*, 1982) directed against ICP8, the major DNA binding of HSV-2, kindly provided by Dr. A. Minson (Department of Pathology, University of Cambridge). After two brief washes in PBS they were incubated with sheep IgG anti-mouse conjugated to fluorescein (Silenus Laboratories Pty Ltd., Melbourne, Australia) for 30 min. The coverslips were washed as before in PBS and briefly counterstained in 0.01% Evan's blue. They were mounted in 50% glycerol in PBS, pH 8.6 and examined using a fluorescence microscope (Zeiss, Melbourne, Australia).

Results and Discussion

Many of the herpesviruses studied to date show a remarkable degree of conservation, even between distantly related herpesviruses, with respect to their glycoprotein B and/or their major DNA binding protein equivalents. This conservation has been found at both the DNA and protein levels (Littler *et al.*, 1981; Yeo *et al.*, 1981; Pellet *et al.*, 1985; Bzik *et al.*, 1986; Cranage *et al.*, 1986; Keller *et al.*, 1986; Balachandran *et al.*, 1987; Gong *et al.*, 1987; Kemble *et al.*, 1987, Maragos and May 1987; Robbins *et al.*, 1987; Stuve *et al.*, 1987; Hammerschmidt *et al.*, 1988; Misra *et al.*, 1988). It was our aim to determine if this conservation extended from a human herpesvirus (HSV-2) to an avian herpesvirus, in this case ILTV, by looking for the ILTV equivalent of the HSV-2 major DNA binding protein (ICP8).

DNA extracted from ILTV strain SA2 was cleaved with *Bam*H1, electrophoresed through a 0.7% agarose gel, transferred to nitrocellulose and probed

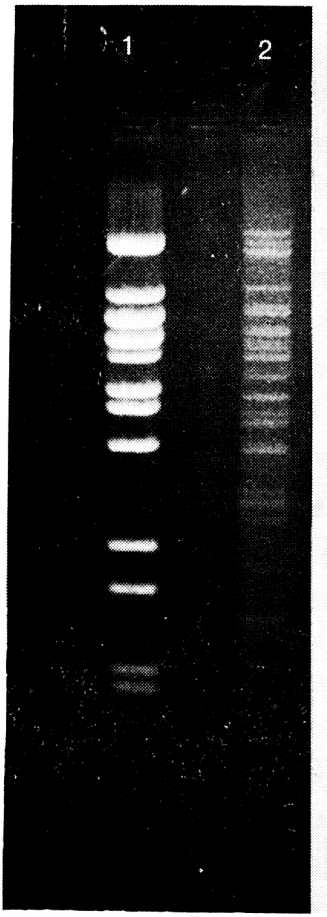

Fig. 1

Restriction endonuclease digest of ILTV DNA and Southern blot hybridization with ^{32}P-labelled HSV-2 *Bgl*II 0 DNA
(1 – 1) Marker DNA consisting of Lambda DNA cleaved with *Bst*EII (New England Bio Labs).
(1 – 2) ILTV DNA cleaved with *Bam*HI and electrophoresed through a 0.7% agarose gel.
(1 – 3) The DNA from 1 – 2 was Southern blotted then probed with ^{32}P-labelled HSV-2 *Bgl*II 0 showing hybridization to the third band in the *Bam*HI profile (approximately 13 kb in size).

with the HSV-2 *Bgl*II 0 DNA fragment (Fig. 1). The HSV-2 *Bgl*II 0 fragment has previously been used to locate the coding region of the bovine herpesvirus type 2 ICP8 equivalent (Maragos and May 1987). The HSV-2 ICP8 containing probe, *Bgl*II 0, hybridized weakly to a single ILTV BamH1 fragment of approximately 13 kb in size (Fig. 1). This result suggests that a possible equivalent region to the HSV-2 ICP8 coding region is present in ILTV DNA.

It is possible that this hybridization may be unrelated to any major DNA binding protein coding sequence and may be an artifact due to the differences in G + C content in the genomes of these two viruses. However, to further support the DNA hybridization results immunofluorescence on ILTV infected and unifected CK cells was performed using a monoclonal antibody directed against the HSV-2 ICP8. Fig. 2 shows the immunofluorescence of monoclonal antibody LP4 on ILTV infected and uninfected CK cells. On virus-unfected cells (Fig. 2-1) there was weak, uniform staining of the nuclei, often without staining of nucleoli. In some nuclei, a very localised accumu-

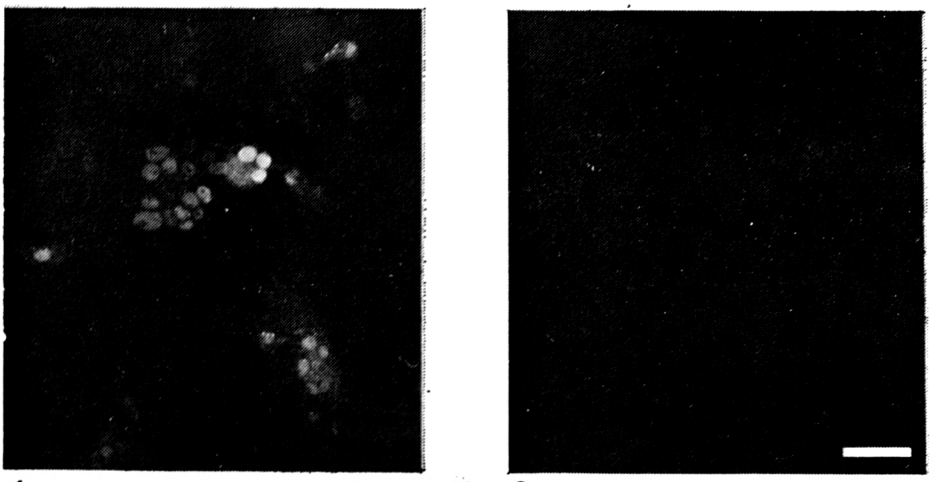

Fig. 2

Immunofluorescent staining of ILT virus-infected (1) and uninfected (2) CK cells with ascites fluid of monoclonal antibody LP4 diluted 1 : 2 0

Bar = 50 μm.

lation of antigen could be seen as small bright dots. There was also a less intense cytoplasmic fluorescence which became more pronouced at later times post-infection. The monoclonal antibody did not produce any fluorescence in uninfected cells (Fig. 2-2). A monoclonal antibody against an unrelated avian virus, infectious bursal disease virus, also gave no fluorescence on either infected or uninfected CK cells (data not shown).

This work further supports the idea that the ICP8 equivalent in herpesviruses is conserved throughout the herpesvirus group. This conservation also appears to include non-mammalian herpesviruses as well as the already well documented mammalian examples. This conclusion is still further supported by the work of Buckmaster *et al.* (1988) who sequenced a large number of DNA fragments from the genomes of Marek's disease virus and herpesvirus of turkeys, then compared the translated amino acid sequence with the amino acid sequences of varicella-zoster virus and human cytomegalovirus. They found several regions of homology, including a region corresponding to their ICP8 equivalents. Unfortunately, since at present there are no restriction enzyme maps available for ILTV, it is not possible to determine whether this cross hybridizing DNA sequence maps to a co-linear position with the reported locations of other herpesvirus ICP8 equivalents.

References

Bagust, T. J. (1986): Laryngotracheitis (Gallid-1) herpesvirus infection in the chicken. 4. Latency establishment by wild and vaccine strains of ILT virus. *Avian Pathol.* **15**, 581 – 595.

Balachandran, N., Oba, D. E., and Hutt-Fletcher, L. M. (1987): Antigenic cross reactions among herpes simplex virus types 1 and 2, Epstein-Barr virus and cytomegalovirus. *J. Virol.* **61**, 1125–1135.

Buchman, T. G., Roizman, B., and Nahmias, A. J. (1979): Demonstration of exogenous genital reinfection with herpes simplex virus type 2 by restriction endonuclease fingerprinting of viral DNA. *J. infect. Dis.* **140**, 295–304.

Buckmaster, A. E., Scott, S. D., Sanderson, M. J., Boursnell, M. E. G., Ross, N. L. J., and M. M. Binns (1988): Gene sequence and mapping data from Marek's disease virus and herpesvirus of turkeys: implications for herpesvirus classification. *J. gen. Virol.* **69**, 2033–2042.

Bzik, D. J., Debroy, C., Fox, B. A., Pederson, N. E., and Person, S. (1986): The nucleotide sequence of the gB glycoprotein gene of HSV-2 and comparison with the corresponding gene of HSV-1. *Virology.* **155**, 322–333.

Cranage, M. P., Kouzarides, T., Bankier, A. T., Satchwell, S., Weston, K., Tomlinson, P., Barrell, B., Hart, H., Bell, S. E., Minson, A. C., and Smith, G. L. (1986): Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccine virus. *EMBO J.* **5**, 3057–3063.

Gong, M., Ouka, T., Matsuo, T., and Kieff, E. (1987): Epstein-Barr virus glycoproteins homologous to herpes simplex virus gB. *J. Virol.* **61**, 499–508.

Hammerschmidt, Conraths, F., Mankertz, J., Pauli, G., Ludwig, H., and Buhk, H.-J. (1988): Conservation of a gene cluster including glycoprotein B in bovine herpes virus type 2 (BHV-2) and herpes simplex virus type 1 (HSV-1). *Virology.* **165**, 388–405.

Keller, P. M., Davison, A. J., Lowe, R. S., Bennett, C. D., and Ellis, R. W. (1986): Identification and structure of the gene encoding gp11, a major glycoprotein of varicella-zoster virus. *Virology.* **152**, 181–191.

Kemble, G. W., McCormick, A. L., Pereira, L., and Mocarski, E. S. (1987): A cytomegalovirus protein with properties of herpes simplex virus ICP8: partial purification of the polypeptide and map position of the gene. *J. Virol.* **61**, 3143–3151.

Kotiw, M., Sheppard, M., May, J. T., and Wilks, C. R. (1986): Differentiation between virulent and avirulent strains of infectious laryngotracheitis virus by DNA: DNA hybridization using a cloned DNA marker. *Vet. Microbiol.* **11**, 319–330.

Kotiw, M., Wilks, C. R., Sheppard, M., and May, J. T. (1984): Comparison of methods for the isolation and purification of infectious laryngotracheitis virus DNA. *Vet. Microbiol.* **9**, 415–419.

Leib, D. A., Bradbury, J. M., Gaskell, R. M., Hughes, C. S., and Jones, R. C. (1986): Restriction endonuclease patterns of some European and American isolates of avian infectious laryngotracheitis virus. *Avian Dis.* **30**, 835–837.

Leib, D. A., Bradbury, J. M., Hart, C. A., and McCarthy, K. (1987): Genome isomerism in two alpha herpesviruses: herpesvirus saimiri-1 (herpesvirus tamarinus) and avian infectious laryngotracheitis virus. *Arch. Virol.* **93**, 287–294.

Littler, E., Yeo, J., Killington, R. A., Purifoy, D. J. M., and Powell, K. L. (1981): Antigenic and structural conservation of herpesvirus DNA binding proteins. *J. gen. Virol.* **56**, 409–419.

Maragos, C., and May, J. T. (1987): Studies of the major DNA binding proteins of two bovine herpes mammillitis virus isolates. *Acta virol.* **31**, 168–171.

McLean, C., Buckmaster, A., Hancock, D., Buchan, A., Fuller, A., and Minson, A. (1982): Monoclonal antibodies to three non-glycosylated antigens of herpes simplex virus type 2. *J. gen. Virol.* **63**, 297–305.

Misra, V., Nelson, R., and Smith, M. (1988): Sequence of a bovine herpesvirus type-1 glycoprotein gene that is homologous to the herpes simplex gene for the glycoprotein gB. *Virology.* **166**, 542–549.

Pellett, P. E., Biggin, M. D., Barell, B., and Roizman, B. (1985): Epstein-Barr virus genome may encode a protein showing significant amino acid and predicted secondary structure homology with glycoprotein B of herpes simplex virus 1. *J. Virol.* **56**, 807–813.

Pignatti, P. F., Cassai, E., Meneguzzi, G., Chenciner, N., and Milanessi, G. (1979): Herpes simplex virus DNA isolation from infected cells with a novel procedure. *Virology* **93**, 260–264.

Rigby, O. W. J., Dieckmann, M., Rhodes, C., and Berg, P. (1977): Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick-translation with DNA polymerase I. *J. molec. Biol.* **113**, 237–251.

Robbins, A. K., Dorney, D. J., Wathen, M. W., Whealy, M. E., Gold, C., Watson, R. J., Holland, L. E., Weed, S. D., Levine, M., Glorioso, J. C., and Enquist, L. W. (1987): The pseudorabies virus gII gene is closely related to the gB glycoprotein gene of herpes simplex virus. *J. Virol.* **61**, 2691–2701.

Sheppard, M., Evans, L., and May, J. T. (1985): Expression of the herpes sinplex virus type 2 major DNA binding protein (ICP8) gene in COS-1 cells. *Virus Res.* **3**, 287–292.

Southern, E. M. (1975): Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. molec. Biol.* **98**, 503–517.

Stuve, L. L., Brown-Shimer, S., Pachl, C., Najarian, R., Dina, D., and Burke, R. L. (1987): Structure and expression of the herpes simplex virus type 2 glycoprotein gB gene *J. Virol.* **61**, 326–335.

Yeo, J., Killington, R. A., Watson, D. H., and Powell, K. L. (1981): Studies on cross-reactive antigens in the herpesviruses. *Virology* **108**, 256–266.

York, J. J., Sonza, S., and Fahey, K. J. (1987): Immunogenic glycoproteins of infectious laryngotracheitis herpesvirus. *Virology* **161**, 340–347.