TISSUE-SPECIFIC RESTRICTION OF LATENT TURKEY HVT TRANSCRIPTION

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Summary. – Using *in situ* hybridization, latent turkey herpesvirus (HVT) transcription was examined in lymphoid and/or nonlymphoid tissues. Blood samples were taken for virus isolation from chickens at 7 and 240 days post infection (PI) representing time points for productive and latent turkey herpesvirus infections, respectively. Spleen, thymus, sciatic and brachial nerves from infected chickens were analyzed for latent HVT transcription and HVT glycoprotein B (gB) expression at 240 days PI. Using indirect immunofluorescence, HVT gB expression was not detected in any tissues examined at 240 days PI. HVT genomic fragments from a HVT *Bam*HI library were used as probes in *in situ* hybridization assays. In the spleen, thymus, sciatic and brachial nerves, latent HVT transcription occurred from the repeat regions flanking the unique long region (TR_L and IR_L). However, fine-mapping of this region revealed a difference in latent HVT transcriptional pattern. A *Sma*I map of the HVT *Bam*HI-F fragment was made to further fine-map latent HVT transcription. A 1.6 kbp *Sma*I subfragment hybridized to cells infected with latent HVT in the spleen and thymus. However, the 1.6 kbp *Sma*I subfragment did not hybridize to cells of the brachial or sciatic nerves. In addition, a 2.0 kbp *Sma*I subfragment hybridized to cells in the thymus but not in the spleen, sciatic or brachial nerves. The above results suggest that latent turkey herpesvirus exhibits tissue-specific transcription.

Key words: HVT; latency; transcription; tissue tropism

Introduction

All three serotypes of Marek's disease virus (MDV) are genomically similar to herpes simplex virus (HSV), an α-herpesvirus. At the molecular level, transcription of α-herpesviruses with type E genome occurs predominantly from the repeat regions flanking the unique long region (TR_L, IR_L) in latent infections (Rock, 1993). In contrast, transcription from latent MDV-1 genome, from a cell line derived from kidney composed of MDV-transformed T lymphoblastoid cells, MDCC-MSB1, maps to the repeat region flanking the unique short region of the genome (IR_S) (Cantello *et al.*, 1994). A MDV-1-specific 10 kb transcript and 2 smaller transcripts are present in the MDCC-MSB1 cells. During productive infection the molecular characteristics of MDV parallel α-herpesviruses. However, the biological features of MDV more closely resemble Epstein-Barr virus (EBV), since both EBV and MDV are lymphotrophic.

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Tissue tropism of latent HSV and EBV infections is similar to productive infections. HSV causes a latent infection in neurons of sensory ganglia (Steiner and Kennedy, 1991). In contrast, γ-herpesviruses such as EBV are predominately lymphotropic. However, EBV also establishes latent infections in stratified squamous epithelium (Brooks *et al.*, 1993).

While low level productive MDV-1 infections appear to persist in the skin and feather tissue (Witter *et al.*, 1971; Buscaglia *et al.*, 1988), latent MDV-1 infections are detected in the spleen, thymus and bursa of infected chickens (Calnek *et al.*, 1981; Shek *et al.*, 1983). In chickens, HVT infects lymphoid, nerve, and feather tissues (Holland *et al.*, 1998). In the present study, we explored the tissue tropism and resulting transcriptional patterns of latent HVT in lymphoid and nerve tissues.

Materials and Methods

Chickens and virus. Chickens were F_1 progeny of line $15I_5$ x 7_1 and were negative for maternal antibodies against HVT or MDV-1. All breeder chickens were housed at the Avian Disease and

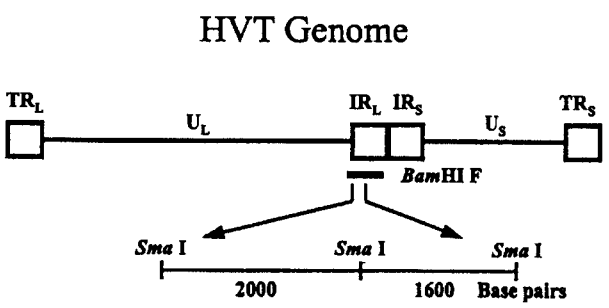

Fig. 1
***Sma*I subfragments of the *Bam*HI-F genomic region**

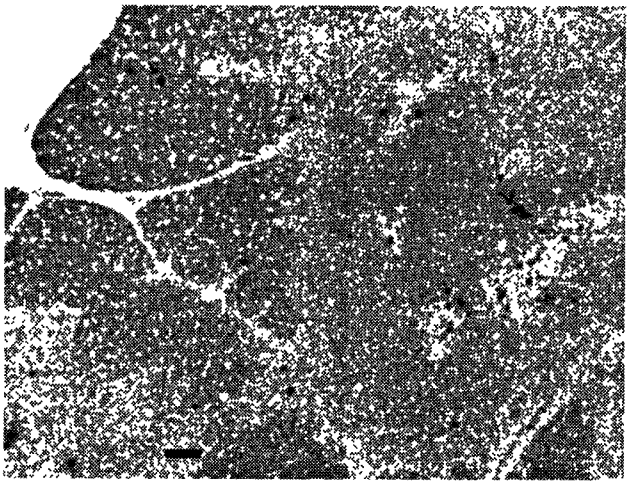

Fig. 2
Photomicrograph of thymus from a chicken infected with HVT 240 days prior to removal of the thymus
In situ hybridization using the *Bam*HI-F fragment as a probe. Note multiple black foci scattered throughout the medulla, Hassall's corpuscles and cortex of the thymus. The foci are representative of presence of latent HVT RNA. Hematoxylin-eosin staining, magnification x100.

Oncology Laboratory and free of antibodies to avian leukosis virus, reticuloendotheliosis virus, and other poultry pathogens. Cell-associated, prototype vaccine virus, FC 126/2 strain of HVT (MDV-3) was maintained in Leibovitz-McCoy's medium supplemented with 1–4% calf serum and antibiotics. Ten chickens were inoculated with 2000 PFU of HVT. Ten chickens were inoculated with Leibovitz-McCoy's medium supplemented with 1–4% calf serum and antibiotics and used as negative controls. Blood samples were collected from chickens at 7 and 240 days PI. Mononuclear cells were separated from serum and red blood cells by centrifugation (IEC model HN-SII, Damon/IEC Division, Needham Heights, MA) at 1000 rpm for 15 mins. The mononuclear cells were resuspended in the supplemented Leibovitz-McCoy's medium and co-cultivated with chicken embryo fibroblasts (CEFs) for virus isolation (Witter *et al.*, 1969). At 240 days PI, chickens were euthanized using a CO_2 gas chamber. The spleen, thymus, sciatic plexus and brachial plexus were transferred to 50 ml conical tubes containing 4% paraformaldehyde. The tissues were placed in tissue cassettes and embedded in paraffin. Five-µm tissue sections were cut from the paraffin blocks and placed on silylated slides which improved adherence of sections to slides. Virus isolation co-cultures were placed on silylated slides and used as positive controls. Uninfected tissues were sectioned as described above and used as negative controls.

In situ hybridization. Probes consisted of a 9.4 kbp HVT *Bam*HI-F fragment, a 1.6 kbp *Sma*I-*Sma*I subfragment of the *Bam*HI-F fragment, and a 2.0 kbp *Sma*I-*Sma*I subfragment (Fig. 1). The 3 fragments have been cloned into pBluescript (Stratagene) and labeled with [^{32}P]dCTP (New England Nuclear Corporation) by random priming (Holland *et al.*, 1998). In addition, *Bam*HI-B fragment, which contains the gene encoding gB, was included as a negative control. All probes hybridized with HVT but not CEFs in Southern blot analysis. The *in situ* hybridization assay has previously been described (Holland *et al.*, 1996).

Indirect immunofluorescence. Monoclonal antibody specific for HVT gB was provided by Lucy Lee, ADOL, USDA-ARS, East Lansing, MI. The indirect immunofluorescence assay has previously been described (Holland *et al.*, 1996).

Results

In situ hybridization of BamHI-F fragment probe to RNA from spleen, thymus, and sciatic and brachial nerves

^{32}P-labeled *Bam*HI-F fragment representing the TR$_L$ and IR$_L$ of the HVT genome hybridized to tissue sections from the spleen, thymus, sciatic and brachial nerves taken from chickens latently infected with HVT. Photographs representative of the *in situ* hybridization are depicted in Fig. 2. Treatment of tissue sections with RNase A prior to *in situ* hybridization resulted in a loss of HVT probe and target RNA hybridization. Therefore hybridization detected HVT RNA but not genomic DNA.

*Bam*HI-F fragment hybridized to all tissue sections infected with latent HVT (Table 1). The probe did not hybridize to uninfected chicken tissues. In addition, the probe hybridized to HVT productively infected CEFs (data not shown). In contrast, the *Bam*HI-B fragment did not hybridize to uninfected tissue sections nor latently HVT-infected tissue sections. The BamHI-B fragment did hybridize to HVT-infected CEFs.

In situ hybridization with SmaI subclones from BamHI-F fragment

The *Bam*HI-F fragment was digested with *Sma*I and two of the subfragments, the 1.6 kbp and 2.0 kbp were cloned into pBluescript KS$^-$. The results of the *in situ* hybridization

Table 1. Mapping of latent HVT transcription within the T_{RL} and IR_L regions flanking the unique long region of HVT genome using *in situ* hybridization

Probe (fragments)	Uninfected controls[a]	Infected controls[a]			
		Spleen	Thymus	Sciatic nerves	Brachial nerves
*Bam*HI-F	0/10	10/10	10/10	8/10	8/10
2.0 kbp *Sma*I	0/10	0/10	9/10	0/10	0/10
1.6 kbp *Sma*I	0/10	8/10	9/10	0/10	0/10
*Bam*HI-B	0/10	0/10	0/10	0/10	0/10

[a]Positive *in situ* hybridization detected in number of chickens/total number examined.

with the two probes are shown in Table 1. The 2.0 kbp *Sma*I subclone (pBSS2) did not hybridize to spleen, sciatic or brachial nerves infected with latent HVT. However, pBSS2 did hybridize to tissue sections from the thymus latently infected with HVT. The 1.6 kbp *Sma*I subclone hybridized to spleen and thymus tissue sections but did not hybridize to sciatic or brachial nerves latently infected with HVT.

Discussion

In our studies, we sought to determine if nononcogenic but lymphotrophic HVT demonstrate differences in transcriptional activity of the latent HVT genome dependent on location of the latent infection. While expression from the 2.0 kbp *Sma*I and 1.6 kbp *Sma*I subfragments was consistently demonstrated in the thymus, only the 1.6 kbp *Sma*I subfragment had transcriptional activity in the spleen, albeit with lower signal intensity than seen in the thymus.

In contrast, neither the 2.0 kbp nor the 1.6 kbp *Sma*I subfragment was expressed in the brachial or sciatic nerves. However, expression of latent HVT *Bam*HI-F fragment did occur in the brachial and sciatic nerves as evidenced by positive *in situ* hybridization. These results emphasize the potential importance of tissue tropism in latent HVT transcription. In addition, the findings reported for HVT latency and transcriptional activity are similar to results seen with HSV.

Previously, we identified the HVT genomic *Bam*HI-F fragment, which encompasses the TR_L and IR_L as being transcriptionally active during HVT latency (Holland *et al.*, 1998). Studies on HSV have revealed that transcription during latency is limited to this same region (Deatley *et al.*, 1987; Puga and Notkins, 1987; Stevens *et al.*, 1987). Earlier reports suggest that common latency regulatory mechanisms occur between various members of α-herpesviruses (Rock *et al.*, 1987). Unlike HSV, two other α-herpesviruses, bovine herpesvirus (BHV) and pseudorabies (PRV) have genomic structures composed of unique long region and unique short region with only the unique short region flanked by inverted repeat regions. For both BHV and PRV latent infections, transcription is limited to the IR_S region, but both these viruses lack TR_L and IR_L regions (Priola *et al.*, 1990; Rock *et al.*, 1987). While MDV-1 has a genomic structure similar to HVT and HSV, transcription in latent viral infections occurs from the IR_S, comparable to BHV and PRV latent transcription (Cantello *et al.*, 1994).

Although HVT and MDV-1 have genomic similarities to type E α-herpesviruses, they both have biological properties comparable to γ-herpesviruses, i.e. EBV, they are lymphotropic. Though both EBV and MDV transform lymphocytes, HVT does not. In EBV latency, 3 different forms exist. Type 1 latency occurs in Burkitt's lymphoma and is characterized by expression of Epstein-Barr nuclear antigen 1 (EBNA1) (Gregory *et al.*, 1990; Rowe *et al.*, 1987). Type II latency is seen in nasopharyngeal carcinomas with the selective expression of EBNA1, latent membrane proteins LMP1 and LMP2 (Young *et al.*, 1988). Type III latency is found in immunoblastic B-cell lymphomas from immunosuppressed individuals and EBV-transformed lymphoblastoid cell lines. All EBNAs, (EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, and EBNA-latent protein), LMP1, and LMP2 are expressed in type III EBV latency (Keiff and Leibowitz, 1990). The three types of EBV latency are transcriptionally distinct (Brooks *et al.*, 1993). In types I and II latency, EBNA1 is expressed from promoter Fp located in the *Bam*HI-F fragment, while LMPs are expressed from different individual promoters in the *Bam*HI-N region. In type III latency, Fp promoter is not active and EBNA mRNA is expressed from either Cp or Wp promoters located in *Bam*HI-C or adjacent *Bam*HI-W region of the genome (Lear *et al.*, 1992; Woisetschlaeger *et al.*, 1989).

Similarly to 3 types of EBV latency, distinct transcriptional patterns of latent HVT exist in different tissues. Although the exact role that tissue tropism plays in latent HVT transcription is still unclear, many interesting possibilities as to the role of tissue tropism in latent HVT transcription exist. One possibility is that cellular factors may be responsible for repressing latent HVT transcription in certain tissues, as seen in HSV latency. In the presence of nerve growth factor (NGF) in primary cultures of sympathetic and sensory neurons, HSV transcription is restricted to TR_L and IR_L. Removal of NGF results in HSV reactivation and corresponding productive HSV transcription (Wilcox and Johnson, 1988; Wilcox *et al.*, 1990; Doerig *et al.*, 1991).

Another prospective role of tissue tropism in latent HVT transcription is maintenance of the HVT genome. In type II latency, expression of EBNA1 is important in EBV genome maintenance. This result underscores the third possibility, that latent HVT encodes proteins necessary for the establishment, maintenance, or reactivation of HVT latency. While the results of this study are truly intriguing, the next logical extension is a more thorough characterization of la-

tent HVT transcription before the functional role of latent HVT transcription in the establishment, maintenance, or reactivation of latent infections can be determined.

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