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Molecular Studies on BIV Infection and its Interaction with Other Bovine Viruses

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Abstract

Bovine immunodeficiency virus (BIV) is a bovine lentivirus that infects animals world-wide. The authors have cloned the BIV env and gag proteins and several sub-unit peptides spanning the entire coding regions of the two proteins as fusions to the bacterial *trp*E protein and expressed them in *Escherichia coli* (Spindler et al. 1984). These recombinant proteins can readily be expressed but the expression levels of these peptides varied. When these proteins were tested against a panel of bovine sera, only sera from BIV-infected animals reacted specifically. One major antigenic determinant was identified in the cartoxyl terminus of the p26 gag protein and another was identified in the transmembrane (TM) domain of the envelope glycoprotein.

The pathogenesis of the BIV infection has not been well characterised. Experimentally-infected animals did not develop immunodeficiency. It is possible that co-factors may play a role in enhancing the pathogenesis of BIV infection; and one of these co-factors could be bovine herpesviruses (BHV). BHV-1 co-infection of BIV-infected cells enhanced BIV replication and an immediate early gene of BHV-1 is involved. This herpes-viral gene product acts on the BIV promotor element to stimulate BIV expression. In vivo co-infection of animals with BIV and BIV-1 demonstrated that these viruses can interact in vivo, with BHV-1 reactivated BIV expression in infected animals.

BOVINE immunodeficiency virus (BIV) is a lentivirus, originally isolated from cattle with lymphocytosis, lymphadenopathy, neuropathy and progressive emaciation (Gonda 1992; Van Der Maaten et al. 1972). However, overt clinical disease in seropositive cattle is rare and the infection is difficult to reproduce experimentally (Carpenter et al. 1992; Flaming et al. 1993; Suarez et al. 1993; Whetstone et al. 1972). BIV antibodies have been detected in beef and dairy cattle in the USA, some European countries, Australia and New Zealand (Amborski et al. 1989, Horner 1991; Horzinek et al. 1991; St. Cyr Coats et al. 1994; Whetsone et al. 1991). However, serological screening of randomly-selected cattle sera for BIV antibody has shown a non-uniform distribution. Sera from eastern or northern parts of the USA are rarely positive for BIV, while approximately 4% of the sera from southern and southwestern regions are BIV positive (Black 1990) and average frequencies with individual herds in the south are considerably higher. For example, 40% of beef and 60% of dairy herds were found to be positive in Louisiana (Gonda 1994), and the seroprevalence of BIV among cattle herds in Mississippi was greater than 50% (St. Cyr Coats et al. 1994).

To screen for naturally-infected cattle, different serological approaches have been employed, including immunofluorescence assays (IFA), Western blot assay (Whetstone et al. 1972, 1991) and enzyme linked immunosorbant assay (ELISA) (St. Cyr Coats et al. 1994). Although these tests were able to detect BIV antibodies using BIV antigen or BIV-infected cells, they lacked sensitivities and required a large mount of native viral antigens. Since BIV can only be propagated well in primary bovine cell cultures and there is a lack of a continuous cell

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line expressing high levels of BIV, the use of native viral proteins for large scale serological testing of BIV is quite difficult. In addition, the IFA and ELISA tests can yield false positive results as compared with Western blot assay (Atkinson et al. 1992). Therefore, there is a need to use recombinant proteins as a source of antigens. Recombinant BIV proteins have been expressed both in bacterial, insect cells and eukaryotic cells (Rasmussen et al. 1990), and the bacterial recombinant gag and env proteins have been used to detect anti-BIV antibodies by Western blot analyses (Atkinson et al. 1992; Chen et al. 1993). These recombinant proteins should be an important source of antigens for large scale seroprevalence screening.

Studies on HIV have demonstrated that several human herpesviruses, such as herpes simplex type 1 (Mosca et al. 1987) and HHV-6 (Horvat et al. 1989), can be potential co-factors in HIV infection and the interactions between HIV and these viruses have been documented. BLV, BVDV, and BHV-1 are common infections in cattle and cause great economic losses to the cattle industry (Cockerell et al. 1992; Gibbs and Rweyemamu 1992; Xue et al. 1990). Studies have demonstrated that BIV and BHV-1 can interact in vitro, similar to what occurred between HIV and human herpesvirus (Geng et al. 1992). An investigation of BIV prevalence and its relationship to other viral infections will provide valuable information in understanding the pathogenesis of BIV infection.

Cloning and Expression of BIV *Gag* and *Env* Genes

BIV-infected animals develop strong antibody responses to various structural proteins like the capsid and the envelope glycoprotein. Antibodies against p26 capsid protein developed earliest (22 days post-infection) and strongest (up to 38 months post-infection) in calves that were experimentally infected by BIV (Whetstone et al. 1990). Therefore, recombinant capsid and envelope proteins can be used as an antigen source to detect animals infected by BIV. The authors used the pATH (protein amendable for making tryptophan hybrid) expression vector which uses the *trp* promoter to express the BIV *gag* and *env* genes. The cloned genes can be expressed as fusions to the trpE protein. The strategy centres on cloning and expression in bacteria of defined regions covering the entire *gag* and *env* coding regions using restriction enzyme deletions of the viral genome. The expressed proteins will then be tested for their reactivities with bovine sera obtained from animals that were infected by BIV. For the *gag* gene, six different clones spanning various regions of the *gag* open reading frame were generated (Fig. 1). These recombinant proteins, especially gag-2, gag-3, gag-4, and gag-5, can be expressed in high quantity and the average yields of the fusion proteins were about 1 mg of 70–80% pure protein harvested from about 200 mL of bacteria. Analysis of the various express gag proteins with a panel of immune bovine sera

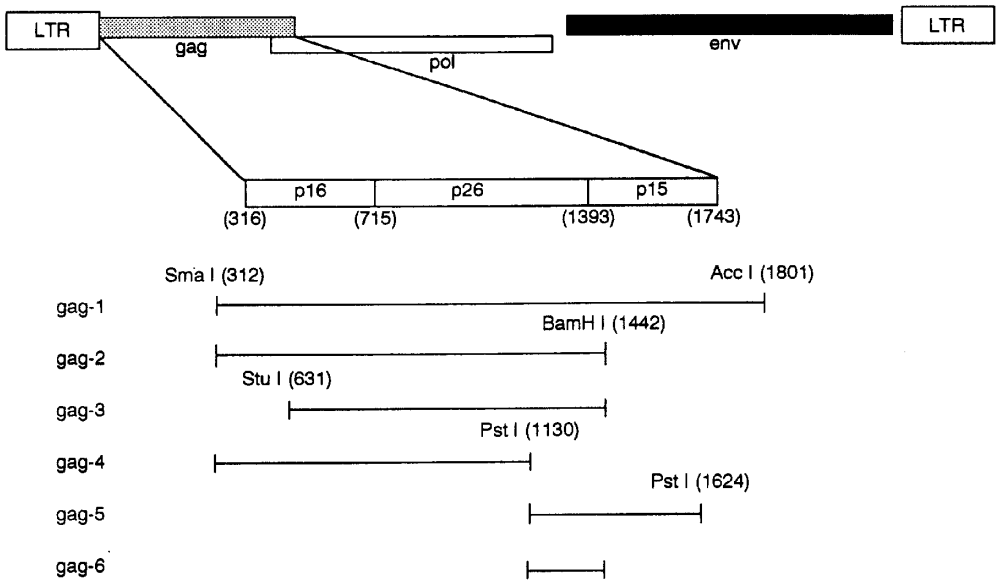

Figure 1. Summary of BIV *gag* constructs. Thick horizontal bars represent the relative genomic organisation of the *gag*, *pol* and *env* coding regions of BIV. Large open box represents the unprocessed $p53^{gag}$ precursor protein with nucleotide positions given below in parentheses. Thin horizontal bars represent coding regions contained within each *gag* construct. Numbers above in parentheses represent the nucleotide positions where specific restriction enzymes cut to generate the specific construct.

enabled identification of at least one major antigenic determinant which can be recognised by all sera tested. This determinant was encoded within the 47 kDa gag protein expressed by the gag-6 construct.

Similar strategy was used to generate nine different recombinant clones that span the various regions of the BIV envelope open reading frame (Fig. 2). These proteins were again expressed as fusions to the trpE protein in *E. coli*. The levels of these expressed proteins varied, some expressed at large quantity that can be detected easily by SDS polyacrylamide gel electrophoresis while others cannot. The recombinant env protein that had the strongest and most consistent reactiion with BIV-infected bovine sera was the protein expressed by env-8. This clone encodes a 134 amino acid peptide that represents the amino terminal region of the transmembrane domain of the envelope glycoprotein. It is interesting that a major antigenic determinant of HIV has also been mapped to a similar location on the HIV-1 gp41 transmembrane glycoprotein (Windheuser and Wood 1988).

Use of Recombinant Proteins to Screen for BIV Seroprevalence

Accumulated serological screening data on BIV seroprevalence in the USA showed a non-uniform distribution (Gonda et al. 1994). Very high seroprevalence was found in the southern United States, e.g., Louisiana and Mississippi (Gonda 1992; St. Cyr Coats et al. 1994), while cattle in the eastern or northern part of the USA are rarely positive. In central USA states, such as Colorado, the seroprevalence rate was about 21%. These differences

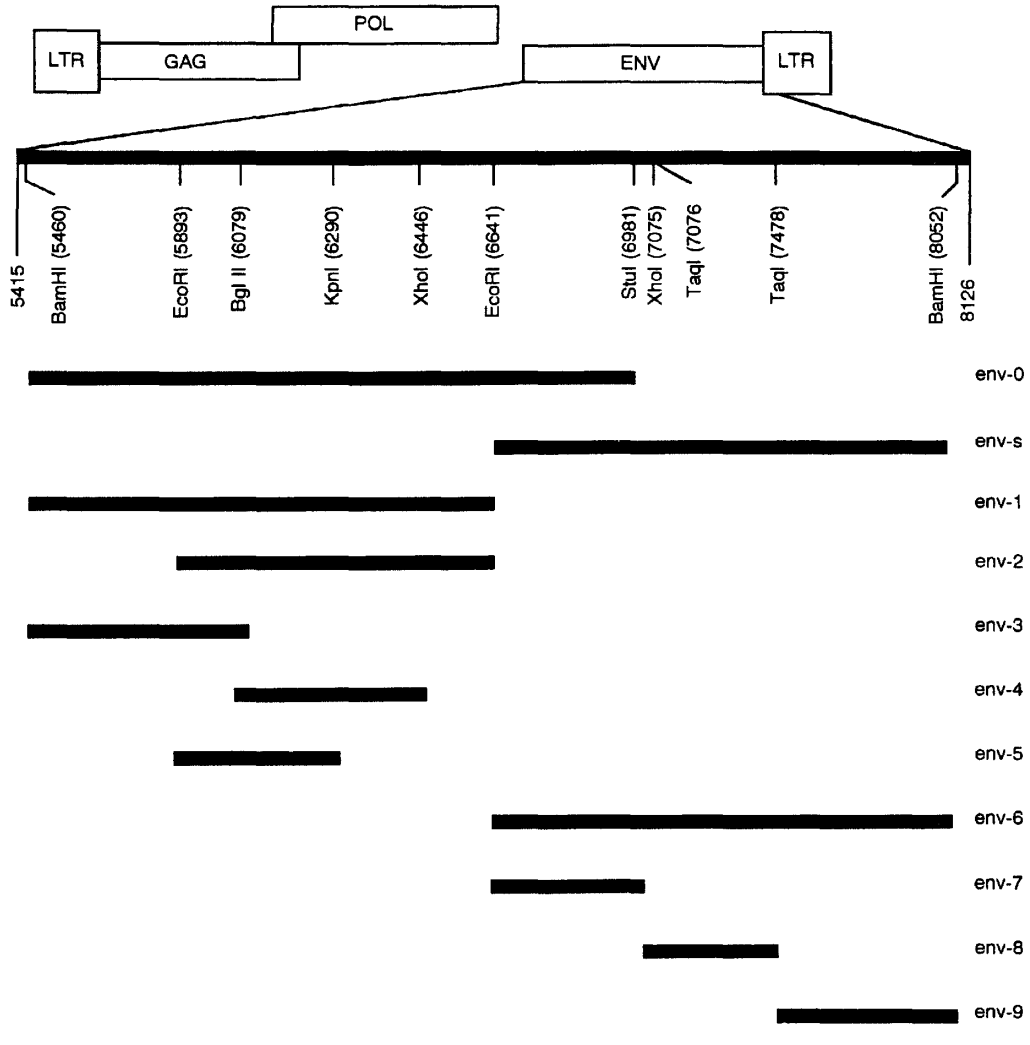

Figure 2. Schematic representation of various inserts of the pATH expression clones which cover most of BIV *env* ORF. The solid line represents the insert of BIV *env* sequence cloned in frame with the *trpE* gene using the indicated restriction enzyme sites. Their nucleotide positions are indicated according to the sequence published by Garvey et al. (1990).

could be due to differences in infection rate or due to the different assays or the source of antigens used in these studies. Because the recombinant BIV proteins can detect BIV antibodies specifically, reproducibly and are extremely sensitive, they were used to survey for the seroprevalence of BIV in cattle from other states in the central USA, such as Kansas. A collection of 155 sera from different parts of Kansas was tested with recombinant BIV gag protein in Western blot assays. Out of the sera tested, 23 were BIV positive (14.84%) (Table 1). The results are consistent with what was observed previously with the Colorado dairy herd based on an ELISA assay (Cockerell et al. 1992).

Table 1. Seroprevalence of BIV in Kansas cattle.

Town or cities	Number tested	Number positive (%)
El Dorado	11	1 (9.08%)
Kansas City A	24	5 (20.8%)
Kansas City B	19	4 (21.05%)
Kansas City C	30	4 (13.33%)
Kinsbury	6	0 (0.00%)
Manhattan	10	1 (10.00%)
Salina	4	1 (25.00%)
Williamsburg	25	5 (20.00%)
Other cities	26	2 (7.69%)
Total	155	23 (14.84%)

Although BIV infections have been reported in the USA, in European countries, Australia and New Zealand, the seroprevalence of BIV in some parts of Asia and of China is not known. In recent years, China has been importing both beef and dairy herds from various European countries and the USA, so it is possible that some of these imported cattle and their progeny may be infected by BIV. It is also possible that domestic herds in China may also be infected by BIV. In order to determine the seroprevalence of BIV, recombinant gag and env proteins were used in Western blot analyses to screen several imported herds and their progeny in the Tianjin area. In addition, domestic herds from several northern provinces of China were also screened. Positive samples were confirmed by PCR analyses using primers that specifically amplify the reverse transcriptase gene of BIV. The screening results are shown in Tables 2 and 3. Of the 689 imported cattle screened from two separate areas, 16 were found to be positive by Western blot and PCR. This represents a 2.32% seropositive rate. To further study the seroprevalence of BIV in other domestic herds in the northern part of China, 754 serum samples were collected from different counties of Zhangjiahou area (Table 3). Twenty-nine samples were found to be positive by Western blot analysis, and confirmed by PCR analyses. The 3–4% seropositive rate is similar to that reported in the south and southwestern regions of the US. However, more samples from various parts of China need to be tested to determine if the seropositive rate varies, as is the case in different geographical locations in the US.

Table 2. Western blot analysis of BIV infection in Tianjin area of China.

Origin of samples	Total number tested	Positive number	Positive rate %
Progeny in Area A	180	4	2.22
Progeny in Area B	509	12	2.36
Total	689	16	2.32

Table 3. Serological survey of BIV in different parts of Zhangjiahou area of China.

Area	Testing number	Positive number	Positive rate
Kangbao County	309	14	4.5%
Chabei Farm	21	2	9.5%
Chongli County	52	2	3.8%
Wei County	132	2	1.5%
Guyuan Farm	76	2	2.6%
Chicheng County	100	4	4.0%
Guyuan County	64	3	4.7%
Total	754	29	3.8%

Interaction between BIV and Bovine Herpesvirus-1 (BHV-1)

Numerous studies (Horvat et al. 1989) have demonstrated that exogenous viral infection, such as human herpesvirus, could be an important co-factor in HIV activation and pathogenesis. It is also possible that animal lentiviruses, like BIV, can also interact with animal DNA viruses to play a role in pathogenesis. These exogenous infections may also be responsible for activation of BIV replication in infected animals. In vitro studies have been conducted to determine the possible interaction between BIV and BHV-1. BIV-infected embryonic bovine lung (EBL) cells were

superinfected with varying doses of BHV-1. Reverse transcriptase (RT) in the culture supernatant was measured to quantitate the levels of BIV expression. Results showed that when BIV-EBL cells were infected with BHV-1, RT activity was detected earlier and at a higher level. The increase is proportional to the amount of herpesviruses added, indicating that there is an increase of BIV expression when herpesviruses were present.

Similar activation of the HIV LTR has been observed with HSV-1, and the IE gene products ICPO and ICP4 have been demonstrated to mediate such activations (Margolis et al. 1989). Because BHV-1 IE gene products BICPO and BICP4 share homology with the HSV-1 IE gene products, tests were conducted to determine if the BHV-1 IE gene can transactivate BIV LTR-directed gene expression.

A p601 fragment was tested that encodes the entire BHV-1 IE gene locus for activation of the BIV-LTR (Fig. 3). When the BHV-1 IE gene fragment p601 was transfected into EBL in the presence of BIV-CAT, only a very moderate (1.7 fold) activation of the BIV LTR was observed. Since p601 fragment contains both BICPO and BICP4 open reading frames, it is possible that they may affect the transactivation function of each other. BICPO and BICP4 were found to share a single promoter, and the mRNAs are generated by differential splicing (Wirth et al. 1991). In order to distinguish their effects on the BIV LTR, partial deletions were made to generate several deletion clones of p601. The p601–D1 has truncated the BICPO gene and p601–D2 has truncation in the BICPO gene (Fig. 3). The results indicated that only p601–D2, which contains an intact BICPO gene, could significantly transactivate BIV LTR. It has thus been shown that the IE gene product BICPO is a potent transactivator of co-infecting viruses like BIV. Since very little is known about the functions of BICPO, its transactivation of the BIV promoter will provide a well-characterised system to study the molecular mechanism involved in BICPO transactivation, whether BICPO acts by binding directly to a viral promoter, or indirectly via other cellular factors.

Due to the in vitro interaction between BHV-1 and BIV, it will be important to determine the seroprevalence of BIV and BHV-1 to determine whether BIV infection could be associated with BHV-1 infection. This study was conducted with 155 serum samples collected from the Kansas herds. In the cases of BIV and BHV-1 antibody detections, 13 of 155 (8.93%) serum samples were both BHV-1 and BIV seropositive. Seventy-five of 155 (48.38%) were BHV-1 seropositive while BIV negative. Statistical results indicated that BHV-1 seroactivities occurred independently of BIV infection (Table 4). There was no association between BIV and BHV-1 ($P>0.05$ in both cases). The results indicate that BIV infection did not clearly affect BHV-1 prevalences and BHV-1 infections did not significantly change the incidence of BIV infection. However, a larger panel of serum samples from different geographical locations should also be tested to determine the relationship between BIV and BHV-1 infection.

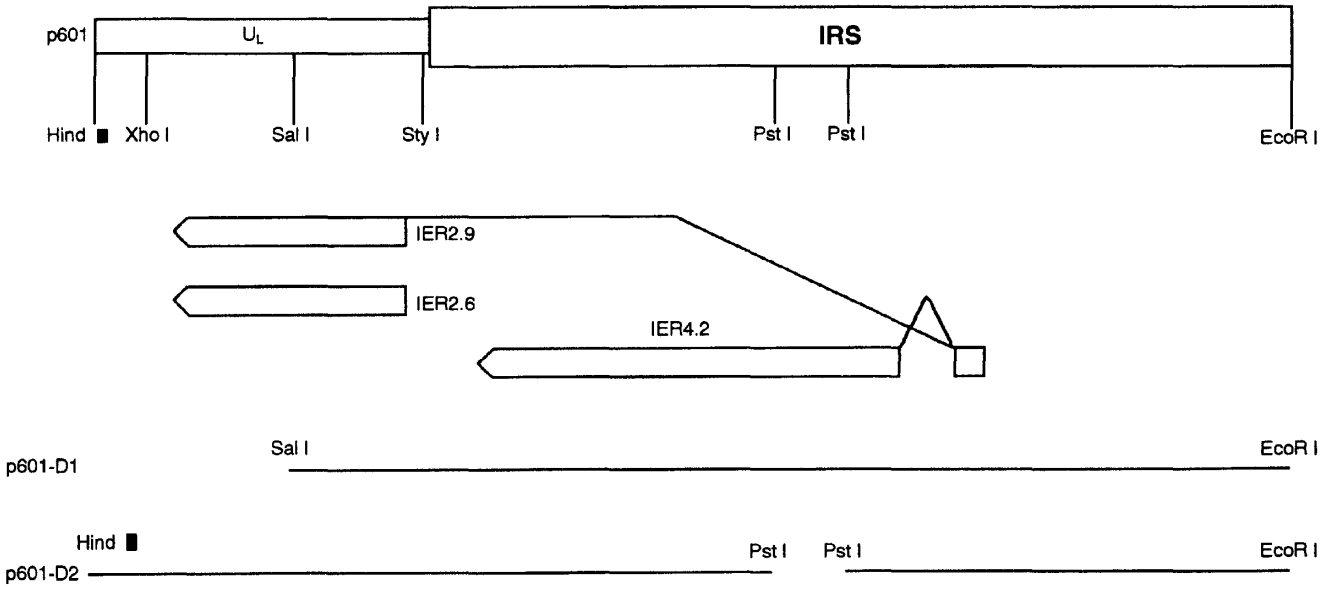

Figure 3. The gene map and partial deletions of p601. Sequences retained in the truncated plasmids are represented by solid lines. p601-D1 contains the complete ORF of IER4.2 (BICP4) and p601-D2 contains the complete ORF of IER2.9 (BICPO).

Table 4. Serological prevalance of BIV and BHV-1.

	BIV positive	BIV negative	Total
BHV-1 positive	13 (8.39%)	75 (48.38%)	88 (56.77%)
BHV-1 negative	10 (6.45%)	57 (36.77%)	667 (43.42%)
Total	23 (14.84%)	132 (85.16%)	155

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