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Production and use of antibodies for the detection of Taura syndrome virus in penaeid shrimp

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ABSTRACT: Monoclonal (MAb) and polyclonal (PAb) antibodies were produced against the penaeid shrimp virus, Taura syndrome virus (TSV), isolated from naturally infected *Penaeus vannamei* from farms in Ecuador, Hawaii and Texas. The PAbs produced in both chickens and mice were capable of detecting TSV in the hemolymph of shrimp during the acute phase of infection by an immunoblot assay. The MAbs were produced using BALB/cByJ mouse spleen cells fused with non-immunoglobulin-secreting SP2/0-Ag-14 mouse myeloma cells. Three MAbs of different immunoglobulin isotypes (IgGγ 1κ, IgGγ 2bκ and IgGγ 3κ) were compared in an immunoblot assay to determine their reactivity to hemolymph from TSV-infected shrimp during the acute and chronic phases of infection and their cross-reactivity, if any, with other shrimp viruses. Western blots of purified TSV were used to compare the specificities of the 3 MAbs for the structural proteins of the virus. The MAbs were used to monitor a laboratory-induced TSV infection in juvenile *P. vannamei*, and they were capable of detecting the virus in the hemolymph of both acute and chronic phase samples. The results with chronic phase hemolymph samples were variable and indicated the need to develop an immunoassay in which the virus in a sample is captured by one antibody and then detected with a second antibody. The availability of MAbs with different specificities for TSV viral proteins and the ability to produce PAbs in chickens will make it possible to develop a sensitive capture assay for rapid detection of the virus in field situations.

KEY WORDS: TSV · Taura syndrome virus · Monoclonal antibodies Immunodetection

INTRODUCTION

Taura syndrome (TS), a serious disease of cultured penaeid shrimp, was first recognized in Ecuador in 1992 (Jimenez 1992, Wigglesworth 1994, Brock et al. 1995) during an epizootic in cultured *Penaeus vannamei*. The causative agent of TS was subsequently found to be a 32 nm icosahedral virus, possessing a single-strand of positive-sense RNA as its genome, and it was tentatively classified as a member of the family *Picornaviridae* (Hasson et al. 1995, Bonami et al. 1997). TS has spread rapidly throughout the Americas affecting farms in Ecuador, Peru, Columbia, Honduras, Guatemala, El Salvador, Brazil, Nicaragua, Belize, Mexico and the United States (Wigglesworth 1994, Lightner 1995, Laramore 1997, Overstreet et al. 1997). Economic losses due to TS epizootics have been devastating to the shrimp farming industry, resulting in hundreds of millions of dollars in lost revenue from this virus alone (Lightner 1995). Different strategies being utilized to circumvent the disease include growing other, less susceptible species of penaeid shrimp, genetic selection of TS-resistant *P. vannamei*, complete dry-out and restocking of affected facilities and regions, and development of reliable, rapid diagnostic tests for the virus. The latter is a crucial component for any successful management program dealing with the virus.

The disease caused by Taura syndrome virus (TSV) has 3 distinct and overlapping phases: acute, transition and chronic (Hasson et al. 1995, Lightner at al. 1995, Hasson 1998). These phases were characterized in detail in *Penaeus vannamei* by Hasson (1998). Briefly, the acute phase of the disease typically occurs 1 to 4 d following initial exposure and can be evident up to 7 d post-exposure in some individuals. It is characterized

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histologically by the presence of multifocal cuticular epithelial lesions in the appendages, gills, hindgut, foregut and general body cuticle which present a 'peppered' or 'buckshot-riddled' appearance due to the induction of pyknotic and karyorrhectic nuclei and spherical cytoplasmic remnants. Up to 90% of an affected population of *P. vannamei* may die during this phase, with the shrimp mostly succumbing to the infection during ecdysis. If shrimp survive the acute phase of the disease, they move into a recovery or transition phase, which is seen in a population of infected shrimp during Days 5 through 8 following initial exposure to the virus. This phase is characterized by the presence of randomly distributed multifocal melanized lesions present in the cuticular epithelium and subcutis, and by the beginning of 'spheroid' formation in the lymphoid organ. If the shrimp successfully undergo another molt, the melanized lesions are cast off with the exuvium and the shrimp enter into the chronic phase of infection. The chronic phase is first seen on Day 6 post-infection and can persist for at least 8 mo (Hasson 1998). Diagnosis of TSV infection during the chronic phase is the most problematic. There are no gross signs of disease and histologically, the only remaining evidence of an infection is the presence of lymphoid organ spheroids, which are not, by themselves, diagnostic for TSV since spheroids can be induced by other pathogens (Owens et al. 1991, Bonami et al. 1992, Nadala et al. 1992, Hasson 1998). Only through the use of *in situ* hybridization with specific gene probes can TSV be conclusively diagnosed in chronic phase shrimp. It is currently not known if shrimp can recover completely from a TSV infection or if they remain chronically infected for life (Hasson 1998).

TSV, purified from infected shrimp tissue, was used to construct a complementary DNA (cDNA) library from which gene probes were developed (Mari et al. 1998). Several of these gene probes have been adapted for use in dot blot and *in situ* hybridization methods (Lightner 1996, Hasson et al. 1997). They have also been used to develop oligonucleotide primers for use in a reverse transcription-polymerase chain reaction (RT-PCR) method (Nunan et al. 1998). The use of *in situ* hybridization using specific cDNA gene probes has been invaluable for characterizing TSV and for detecting the virus in chronically infected shrimp. The RT-PCR method has proven to be a sensitive technique for detection of the virus in hemolymph samples during all stages of the disease although the method has not yet been adapted for use with tissues or fixed samples. These molecular methods for detection of TSV are sensitive and specific; however, because they are expensive to perform and because single-stranded RNA can be rapidly degraded by ubiquitous enzymes as well as by hydrolysis at acid or alkaline pH, the successful application of cDNA gene probes and RT-PCR has been limited to controlled laboratory conditions using special sample preparation methods.

An alternative to the use of gene probes and PCR for virus detection in shrimp is utilization of specific antibodies directed against the relatively stable protein matrix of the viral capsid. This report describes the production of polyclonal (PAb) and monoclonal (MAb) antibodies to TSV, and the application of serological methods for rapid and sensitive detection of the virus in hemolymph from infected shrimp.

MATERIALS AND METHODS

Antigens. TSV used for immunizations was purified from naturally infected *Penaeus vannamei* tissue according to the procedure of Bonami et al. (1997) and was a pool of purified virus originating from 3 different geographic regions, Ecuador (Ec), Hawaii (HI) and Texas (TX). TSV used for antibody testing was purified from laboratory-induced or naturally infected *P. vannamei* tissue. The isolates from different geographic regions were not pooled for testing of the antibodies. Purity of the virus preparations was confirmed by transmission electron microscopy (TEM) using 2% phosphotungstic acid (PTA) as the negative stain and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Infectious hypodermal and hematopoietic necrosis virus (IHHNV) and hepatopancreatic parvo-like virus (HPV), which were used to determine antibody specificity, were purified from infected shrimp tissue according to published procedures (Bonami et al. 1990, 1995). Hemolymph, collected from the ventral sinus using 10% sodium citrate to prevent coagulation, was obtained from shrimp infected with TSV, IHHNV, white spot syndrome virus (WSSV) and yellow head virus (YHV), and from specific pathogen-free (SPF) *P. vannamei* (Wyban 1992), which were negative for all of the above viral pathogens. The disease status of the shrimp was confirmed by routine hematoxylin and eosin staining of fixed tissues (Lightner 1996).

Polyclonal antibody production. Three week old white leghorn chickens were injected subcutaneously with purified TSV emulsified in the synthetic adjuvant LES+STM™ (RIBI Immunochem Research, Inc., Hamilton, MT). Five week old BALB/cByJ mice (Jackson Laboratories, Bar Harbor, ME) were injected intraperitoneally with purified TSV emulsified in the synthetic adjuvant MPL+TDM™ (RIBI Immunochem Research, Inc.). The sera were collected after 3 to 5 booster immunizations at 14 to 21 d intervals.

Monoclonal antibody production. Spleen cells were obtained from 1 of the mice used for PAb production 2 d after the third TSV immunization. SP2/0-Ag-14 myeloma cells (American Type Culture Collection, Rockville, MD), pretreated with 8-azaguanine (Sigma Chemical Co., St. Louis, MO), were fused with the mouse spleen cells using polyethylene glycol 4000 (gas chromatography grade; Curtin Matheson Scientific, Houston, TX) according to previously described protocols (Kohler & Milstein 1976, Kearney et al. 1979, Oi & Herzenberg 1980). The resultant hybridoma cell cultures were tested for the production of immunoglobulin G (IgG) antibodies specific for TSV by dot blot immunoassay. Positive hybrids were selected and cloned 3 or more times by limiting dilution (Galfre & Milstein 1981). Monoclonal antibodies were harvested from hybridomas grown in protein-free hybridoma medium (PFHM-II; Life Technologies, Gibco BRL, Grand Island, NY) for 7 to 10 d at 37°C with 5% CO_2. The MAbs produced in PFHM-II were concentrated 8 to 10 times using Centriprep-30™ centrifugal concentrators (Amicon, Inc., Beverly, MA). They were isotyped using the IsoStrip™ mouse monoclonal antibody isotyping kit (Boehringer Mannheim Corp., Indianapolis, IN) according to the manufacturer's directions.

Dot blot immunoassay. Antibodies were assayed by dotting 1 µl of test antigen (purified virus, infected shrimp hemolymph or SPF shrimp hemolymph) onto the surface of MA-HA-N45 assay plates (Millipore, South San Francisco, CA). After air drying, the wells were blocked for 1 h at room temperature (RT) with 200 µl of a buffer containing phosphate buffered saline and 0.05% Tween 20 (PBST) mixed with 10% normal goat serum (Life Technologies, Gibco BRL) and 2% Hammersten casein (Amersham Life Sciences, Arlington Heights, IL). The wells were washed 3 times with PBST and were then reacted with 100 µl primary antibody (MAb or mouse PAb) for 30 min at RT. Alkaline phosphatase (AP) labeled goat anti-mouse IgG, γ chain specific, secondary antibody (Zymed, South San Francisco, CA) diluted 1:1000 in PBST plus 10% normal goat serum was used for detection (30 min, RT). After washing 3 times with PBST, 1 time with PBS and 1 time with distilled water, the reactions were visualized by development for 15 min at RT with nitroblue tetrazolium and bromo-chloro-indoyl phosphate (Boehringer Mannheim Corp.) in Tris-NaCl (100 mM each) buffer containing 50 mM $MgCl_2$, pH 9.5. Reactions were stopped with distilled water. In assays employing the chicken PAb, an AP-labeled rabbit anti-chicken IgG (Sigma Chemical Co.), diluted 1:1000, was used as the secondary antibody. The reactions were graded such that a maximum +4 intensity was equivalent to the reaction generated using the mouse anti-TSV PAb at a dilution of 1:1000 against purified TSV or infected hemolymph. A negative reaction was one in which no colored spot was visible in the well. Normal mouse or chicken serum was used as a negative control for the primary antibody.

Western blot analysis. Structural proteins of TSV purified from individual geographic isolates were denatured in Laemmli buffer (Laemmli 1970) containing 10 M urea and were separated using 12% SDS-PAGE (Bonami et al. 1997). The proteins from the HI isolate of TSV were electro-transferred to nitrocellulose membranes using 0.5× Towbin transfer buffer (Towbin et al. 1979) for 3 h using 300 mA constant current. The membranes were reacted with specific MAbs obtained from hybridoma cell culture fluids or with mouse PAb diluted 1:1000 and the reactions were visualized as in the dot blot immunoassay. A marker, containing proteins of the following molecular weights, was run in the SDS-PAGE for reference: 97.4, 66.2, 39.2, 26.6, 21.5 and 14.4 kDa (Boehringer Mannheim Corp.).

Detection of TSV in shrimp hemolymph. Experimental TSV infections were induced in SPF *Penaeus vannamei* obtained from the Oceanic Institute in Honolulu, HI (Wyban 1992). These shrimp were negative for all known viral pathogens of shrimp. A tissue homogenate was prepared from naturally infected TSV-infected shrimp carcasses (originating from HI) by grinding 1 g of tissue in 2 ml buffer containing 20 mM Tris-HCl and 400 mM NaCl, pH 7.4, followed by centrifugation to pellet out the solid debris. The tissue homogenate was diluted 1:10 in 2% saline just prior to injection of SPF *P. vannamei* into the tail muscle. Hemolymph was collected from the ventral sinus of moribund shrimp in the acute phase of infection or from shrimp in the chronic phase. Experimental shrimp were maintained in the laboratory using modifications of the methods described in Williams et al. (1992). Shrimp were held in 30 l glass aquaria with prepared sea water (Forty Fathoms Bio-Crystals Marinemix, Marine Enterprises International, Inc., Baltimore, MD) at 24 to 26 ppt salinity, 28°C. The aquaria were fitted with a biological filter containing crushed oyster shells and granulated carbon. Water was recirculated through the filter by an airlift. Aeration for the air lifts and the water was generated by an electric air pump and delivered to the tanks with Teflon air lines.

RESULTS

Antigen purification

TSV used for antigen was purified from naturally infected shrimp tissue originating from 3 geographic regions: Ec, HI and TX. TSV from the 3 regions was

pooled for immunization. Purified virus preparations were examined by TEM after negative staining with 2% PTA and were determined to be free of other infectious agents (data not shown; Hasson et al. 1995 and Bonami et al. 1997 for representative TEMs of TSV). Analysis of SDS-PAGE gels of purified TSV preparations revealed only the presence of 1 minor (58 kDa) and 3 major (24, 40 and 55 kDa) protein bands which are characteristic for TSV (Bonami et al. 1997). Fig. 1 shows the SDS-PAGE gel for the HI isolate of TSV. There were no differences seen in the electrophoretic patterns of TSV from HI, Ec or TX (data not shown).

Antibody titrations

Antibody titers were determined by dot blot immunoassay against purified TSV (HI isolate), TSV-infected hemolymph (HI isolate) and SPF hemolymph from uninfected *Penaeus vannamei*. The chicken and mouse PAbs displayed weak cross-reactivity to SPF hemolymph which was evident only at dilutions below 1:250 (Table 1). The mouse PAb had a much higher titer against purified TSV and TSV-infected hemolymph than did the chicken PAb (Table 1). Three mouse MAbs, each with a different immunoglobulin isotype, were chosen for characterization. The titers of the MAbs produced in PFHM-II medium were determined both before and after concentration against purified TSV, TSV-infected hemolymph, and SPF hemolymph. As shown in Table 1, MAb 1 consistently had the highest titer against TSV antigens and demonstrated no cross-reactivity to SPF hemolymph. MAb 2 and MAb 3 had lower titers than MAb 1 to TSV antigens but were also not cross-reactive with SPF hemolymph. Concentration of the MAbs resulted in increased titers against TSV antigens, with no increase in reactivity to SPF hemolymph (Table 1).

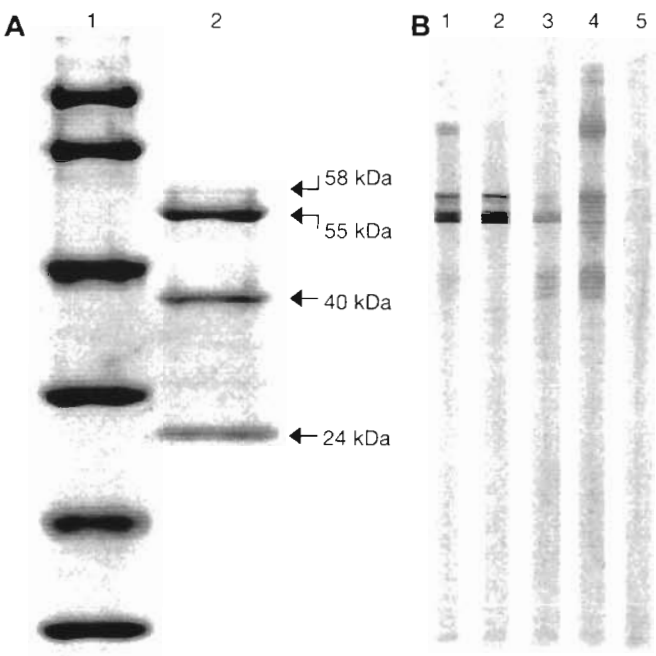

Fig. 1. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and (B) Western blot of purified TSV (HI). (A) SDS-PAGE gel stained with Coomassie blue: lane 1 contains molecular weight markers with bands at 97.4, 66.2, 39.2, 26.6, 21.5 and 14.4 kDa; lane 2 contains polypeptides from purified TSV. (B) Western blot of purified TSV polypeptides: lane 1 reacted with mouse polyclonal antibody diluted 1:1000; lane 2 reacted with monoclonal antibody (MAb) 1 (γ1κ); lane 3 reacted with MAb 2 (γ2bκ); lane 4 reacted with MAb 3 (γ3κ); lane 5 reacted with secondary antibody only

Antigen specificity of MAbs

In order to confirm the specificity of the MAbs for TSV, they were tested by dot blot immunoassay against other purified viruses of penaeid shrimp (IHHNV and HPV) and against hemolymph from shrimp infected with other shrimp viruses (IHHNV, WSSV and YHV). As shown in Table 2, MAbs 1 and 2 were specific for TSV and TSV-infected hemolymph, whereas MAb 3 demonstrated cross-reactivity to hemolymph from IHHNV-, YHV- and WSSV-infected shrimp. The MAbs reacted to purified TSV from all 3 geographic regions, HI, Ec and TX. A secondary antibody control, in which PFHM-II medium without any MAb was used in place of the primary antibody, demonstrated no background reactions to the antigens tested.

Western blot analysis of mouse antibodies

Western blots were performed only with the HI isolate of TSV In Western blots, the mouse PAb (1:1000) reacted strongly to the 55 and 58 kDa TSV polypeptides (Fig. 1B, lane 1). The Western blot showed a faintly reacting, discreet band located just below the 58 kDa region. This faint band was not noted in Coomassie stained gels and its significance is unknown. A very weak reaction to the 40 kDa polypeptide was also noted which was visible at a dilution of 1:500 (not shown). The PAb also displayed a weak reaction to an additional band not seen in the Coomassie stained gel which was approximately 70 kDa in size. The MAbs demonstrated differences in their polypeptide binding patterns. MAb 1 (Fig. 1B, lane 2)

Table 1. Titer of polyclonal (PAb) and monoclonal antibodies (MAb) to TSV (HI), before and after concentration, as assessed by dot blot immunoassay. Concentrated MAbs were processed as described in the text and were 3 to 4 times more concentrated than the original hybridoma cell culture fluids. The titer is the reciprocal of the highest dilution giving a +3 or greater reaction

Antibody	Purified TSV	TSV + hemolymph	TSV – hemolymph
Chicken PAb	2000	500	<250
Mouse PAb	32000	8000	<250
MAb 1 (γ1κ)	512	256	<2
MAb 2 (γ2bκ)	64	16	<2
MAb 3 (γ3κ)	128	128	<2
MAb 1 concentrated	16384	8196	<4
MAb 2 concentrated	1024	64	<4
MAb 3 concentrated	1024	1024	<4

reacted strongly to the 55 and 58 kDa polypeptides. It also showed a faint reaction to the band located just below the 58 kDa polypeptide which was not evident in Coomassie stained gels. MAb 2 (Fig. 1B, lane 3) demonstrated a moderate reaction to the 55 kDa TSV polypeptide, a weak reaction to the 40 kDa polypeptide and a barely visible reaction to the 58 kDa polypeptide. MAb 3 (Fig. 1B, lane 4) reacted weakly to the 55 kDa TSV polypeptide and moderately to the 58 and 40 kDa TSV polypeptides. It also reacted to the 70 kDa band and to the band located just below the 58 kDa polypeptide which were not seen in Coomassie stained gels (Fig. 1A, lane 2). With MAb 3, a reaction was also noted at the top of the membrane which corresponded to the top of the resolving gel and was presumably protein that was too large to migrate in the gel during electrophoresis. Neither the MAbs nor the PAb reacted with the 24 kDa polypeptide. The secondary antibody did not display reactivity to any of the polypeptides.

Table 2. Specificity of anti-TSV MAbs assessed by dot blot immunoassay. Reactions were scored on a scale of increasing intensity (+1 to +4). TSV negative (–) hemolymph was obtained from specific pathogen-free shrimp

Antigen	MAb 1 (γ1κ)	MAb 2 (γ2bκ)	MAb 3 (γ3κ)
Purified TSV (Ec)	+3	+3	+3
Purified TSV (HI)	+4	+3	+3
Purified TSV (TX)	+3	+3	+3
TSV + hemolymph (HI)	+4	+3	+4
TSV – hemolymph	–	–	–
Purified IHHNV	–	–	–
IHHNV + hemolymph	–	–	+2
Purified HPV	–	–	–
YHV + hemolymph	–	+1	+2
WSSV + hemolymph	–	–	+4

Detection of TSV in shrimp hemolymph

Hemolymph was obtained from shrimp during the acute (1 to 5 d post-infection) and chronic (6 to 10 d post-infection) phases of a TSV infection. For the purposes of this study, no distinction was made for the transition phase and shrimp in this phase were grouped with the chronic phase shrimp. The samples were tested by immunoblot assay using MAb 1 as the primary antibody. MAb 1 was capable of detecting TSV in all of the samples during the acute phase with the majority of reactions (21/24) ranging from +2 to +4 (Table 3). During the chronic phase, MAb 1 was also able to detect TSV in all of the samples, but the reactions were less intense and more variable with the majority of the reactions (14/16) ranging from +1 to +3 intensity (Table 3).

DISCUSSION

The primary purpose of this study was the development of highly specific monoclonal antibodies to TSV which can be produced economically and used to develop rapid immunoassays for detection of the virus in field situations. Three different mouse hybridoma cell lines were obtained which produce monoclonal antibodies against TSV and which have different immunoglobulin isotypes. The IgG γ1κ isotype MAb (MAb 1) consistently demonstrated the highest titer and the most specific reactions when compared with the IgG γ2bκ isotype MAb (MAb 2) and the IgG γ3κ isotype MAb (MAb 3). All 3 of the MAbs reacted with more than a single band in Western blots. MAb 1 reacted strongly with the 58 and 55 kDa TSV structural

Table 3. Summary of dot blot immunoassay results of hemolymph samples from *Penaeus vannamei* infected with TSV (HI) and reacted with monoclonal antibody MAb 1 (γ1κ). Reactions were scored on a scale of increasing intensity (+1 to +4). Acute phase samples were collected 1 to 5 d post-infection and chronic phase samples were collected 6 to 10 d post-infection with TSV

Immunoblot reaction	Acute phase (No. reacting/total)	Chronic phase (No. reacting/total)
Negative	0/24	0/16
+1	3/24	5/16
+2	8/24	4/16
+3	4/24	5/16
+4	9/24	2/16

proteins by Western blot analysis, whereas MAb 2 reacted with only moderate intensity to the 55 and 40 kDa structural proteins. MAb 3 reacted with the 58, 55 and 40 kDa TSV proteins as well as to other bands which were not evident in the stained gel. The PAb and 2 of the MAbs (1 and 3) also reacted very faintly to bands not seen in Coomassie stained gels. The significance of these bands is not known. They may be TSV proteins that occur in very low concentrations and are therefore not evident by Coomassie blue staining, or they may be shrimp proteins which co-purified with the virus. Regardless, they were present in the antigen preparation used for immunization since the PAb demonstrated reactivity to them by Western blot analysis. The lack of reactivity to the 24 kDa polypeptide by any of the MAbs or the mouse PAb indicates that this protein is not highly immunogenic in mice. The chicken PAb also did not react to the 24 kDa polypeptide (data not shown).

The multiple reactivities seen with the MAbs in the Western blots may have several explanations. In the case of MAb 1, the antibody was shown to be highly specific for TSV by immunoblot reactions and it was produced from a stable hybridoma that had been subcloned 7 times before the antibody was harvested for Western blot analysis. It is likely that the reaction to the 55 and 58 kDa bands is due to the presence of common epitopes in these 2 capsid proteins. There are examples of proteolytic breakdown of polypeptides in other picorna-like viruses in which the resulting capsid proteins have identical stretches of amino acid sequences, but differ in their length. This is best exemplified by the capsid proteins of the aphid picorna-like virus, *Acyrthosiphon pisum* virus (APV; Van Der Wilk et al. 1997). In APV, the 4 capsid proteins all share an identical 16 amino acid sequence. Based on analysis of the DNA and amino acid sequence data, the authors conclude that the 23/24 kDa protein is a proteolytic breakdown product of the 34 kDa protein, whereas the 66 kDa protein is the result of a translational frameshift which occurs downstream of the region of amino acid identity of the capsid proteins. In another example, an insect virus in the Nodaviridae family exhibits spontaneous cleavage of the major capsid protein in the mature virion (Schneemann et al. 1992). In SDS-PAGE gels of mature virions, the precursor protein is a minor band only slightly larger than the major capsid protein. The small protein cleaved from the precursor is only 5 kDa in size, stains poorly with Coomassie brilliant blue and is often not visible in the gels. The presence of a common epitope may also explain the reactivity of MAb 2, which reacted to the 55 kDa and 40 kDa capsid proteins in Western blots and also demonstrated specificity for TSV in immunoblot tests. Conclusive evidence for the presence of identical amino acids shared by the 58, 55 and 40 kDa capsid proteins must await final DNA and amino acid sequence analyses of TSV, which is in progress by Dr Jocelyne Mari in our laboratory at the University of Arizona (pers. comm.).

In the case of MAb 3, numerous bands reacted in the Western blot and all of them were of low or moderate intensity. Considering the data from the immunoblots where MAb 3 cross-reacted with hemolymph from shrimp infected with other viruses, the results indicate that MAb 3 is probably a low avidity antibody which results in non-specific binding. If this is correct, then MAb 3 will not be a useful antibody for the development of specific test formats for TSV.

The TSV used in Western blots was purified from tissue originating in HI. Future work will determine if the same reaction pattern is seen with TSV purified from tissue originating in Ec and TX since a pool of these 3 geographic isolates was used to immunize the mice for the production of the hybridomas. In the characterization of the viral agent, Bonami et al. (1997) found the Ec and HI TSV isolates to be identical in their biophysical, biochemical and biological characteristics. MAb 1 can be used to assist in determining if TSV isolates from these different geographic regions are also serologically identical.

TSV has been suggested for inclusion in the family *Picornaviridae* (Hasson et al. 1995, Bonami et al. 1997). It is like other picornaviruses with respect to the size, shape and buoyant density of the viral particle and with respect to the type and size of the genome (Murphy et al. 1995). However, TSV is unique from other picornaviruses in the number and sizes of the structural proteins as determined by SDS-PAGE analysis: only 3 major capsid proteins have been identified in TSV which are seen in approximately equal molar ratios (Bonami et al. 1997). The Western blot reactivity of MAb 1, and possibly MAb 2, indicate that there may be some regions of amino acid similarity in the capsid proteins which would further differentiate TSV from mammalian picornaviruses, but which would align it more closely with other picorna-like viruses of insects.

Since MAb 1 demonstrated the strongest and most specific reactivity to TSV, it was employed to assess the efficacy of using a direct immunoblot assay for detection of TSV in acute and chronic phase hemolymph samples. The results demonstrate that the direct immunoblot assay is sufficient for detection of TSV in acute phase hemolymph samples, but that it is not sensitive enough for consistent detection of the virus during the early chronic phase of infection. Using TSV purified from infected shrimp tissue, it was also possible to produce polyclonal antibodies to the virus. Polyclonal antibodies were produced in both chickens and mice. The availability of chicken anti-TSV antibodies offers potential for development of antigen

'capture' assays in which a second antibody may be employed to detect the captured virus. This is currently being investigated as a way to provide for more sensitive detection of TSV from chronic phase hemolymph.

The data presented here demonstrate the specificity of MAbs and PAbs produced to the penaeid shrimp virus TSV. Furthermore, the results indicate that MAb 1 will be useful in the development of other serological test formats such as immunofluorescent antibody tests that will permit the rapid detection of TSV in shrimp tissue or hemolymph. Recently, Luis Matheu (Universidad del Valle de Guatemala, pers. comm.) tested MAb 1 on TSV-infected fixed tissue sections using a goat anti-mouse AP conjugate for final detection and demonstrated reaction patterns that matched serial sections which had been tested with a TSV specific gene probe. The data reveal that antibodies produced to TSV can be employed for sensitive *in situ* histological analysis and may provide tools for further study of TS viral pathogenesis in penaeid shrimp. It may also be possible to utilize these antibodies for purification of virions from infected samples or for purification of structural viral proteins by attachment of the antibodies to an affinity column matrix.

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