JOURNAL OF VIROLOGY, Jan. 2003, p. 1638–1643 Vol. 77, No. 2
0022-538X/03/$08.00+0 DOI: 10.1128/JVI.77.2.1638–1643.2003

Hantavirus Pulmonary Syndrome-Associated Hantaviruses Contain Conserved and Functional ITAM Signaling Elements

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Received 10 June 2002/Accepted 10 October 2002

Hantaviruses infect human endothelial and immune cells, causing two human diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). We have identified key signaling elements termed immunoreceptor tyrosine-based activation motifs (ITAMs) within the G1 cytoplasmic tail of all HPS-causing hantaviruses. ITAMs direct receptor signaling within immune and endothelial cells and the presence of ITAMs in all HPS-causing hantaviruses provides a means for altering normal cellular responses which maintain vascular integrity. The NY-1 G1 ITAM was shown to coprecipitate a complex of phosphoproteins from cells, and the G1 ITAM is a substrate for the Src family kinase Fyn. The hantavirus ITAM coprecipitated Lyn, Syk, and ZAP-70 kinases from T or B cells, while mutagenesis of the ITAM abolished these interactions. In addition, G1 ITAM tyrosines directed intracellular interactions with Syk by mammalian two-hybrid analysis. These findings demonstrate that G1 ITAMs bind key cellular kinases that regulate immune and endothelial cell functions. There is currently no means for establishing the role of the G1 ITAM in hantavirus pathogenesis. However, the conservation of G1 ITAMs in all HPS-causing hantaviruses and the role of these signaling elements in immune and endothelial cells suggest that functional G1 ITAMs are likely to dysregulate normal immune and endothelial cell responses and contribute to hantavirus pathogenesis.

Hantaviruses are enveloped negative-stranded RNA viruses with a tripartite genome and comprise a distinct genus within the family *Bunyaviridae* (47). Hantaviruses infect human endothelial cells and immune cells causing two human diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) (34, 36, 40, 54, 61, 63). Although hantavirus diseases are associated with significant human mortality rates, rodent or small mammal hosts are persistently infected with no apparent deleterious effects (47). Similarly, infection of human endothelial cells is not associated with apoptotic or lytic effects, and there is little immune cell recruitment to the endothelium of infected patients (25, 36, 54, 61, 63). Currently there is little understanding of how hantaviruses regulate immune or endothelial cell function and effect pathogenic responses.

Immune cells are activated by ligands binding to cell surface receptors. Engagement of B-cell (BCR) and T-cell receptors (TCR) directs intracellular signaling responses which result in cellular activation or proliferation. BCR and TCR contain conserved immunoreceptor tyrosine-based activation motifs (ITAMs) within their cytoplasmic tails, and these elements convey extracellular signals to intracellular signaling pathways (2, 7, 42, 56). ITAMs consist of two tandem Yxx(L/I) sequences that are required to direct intracellular signaling responses initiated by a family of receptors (2, 42, 43). ITAM-containing receptors include TCR-ζ, immunoglobulin α (Igα), Igβ, CD3γ, CD3δ and FcεRIγ in immune cells (2, 3, 12, 19, 55). Following ligand binding, Src family kinases phosphorylate BCR and TCR ITAM tyrosines (4, 20). This in turn recruits Syk family kinases to the paired phosphorylated tyrosines and activates downstream signaling pathways (4, 6, 7, 20, 43, 57). Phosphatases recruited to ITAMs also down regulate receptor signaling responses (23, 38, 41). Recently, Syk interactions have also been shown to play critical roles in endothelial cell function (18, 60). These findings indicate that ITAM-directed signaling occurs in several cell types that are infected by hantaviruses.

Viruses that mimic or regulate ITAM signaling responses have also been found to contain ITAMs. ITAMs are present in the gp30 of bovine leukemia virus, LMP2A of the Epstein-Barr virus (EBV), Nef from simian immunodeficiency virus (SIV), and the K1 protein of Kaposi's sarcoma-associated herpesvirus (KSHV) (1, 8, 24, 27, 31, 58). Human immunodeficiency virus (HIV) Nef also forms complexes with TCR ITAMs to regulate immune cell signaling (59). The presence of an ITAM in SIV Nef causes the virus to use macrophages as a primary viral reservoir and is a determinant of acute pathogenesis when present in SIV variants (8, 27, 46). Although the means by which these interactions contribute to pathogenesis are not completely understood, they provide a central means for viruses to modify immune and endothelial cell responses. As a result, viral ITAMs have the potential to play key roles in viral pathogenesis by regulating viral clearance, immune cell activation, immune cell recruitment, and changes in vascular permeability.

The hantavirus M segment encodes two surface glycoproteins, G1 and G2, and the G1 protein contains a long cytoplas-

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mic tail (152 residues). In this report, we show that all HPS-causing hantaviruses contain a conserved ITAM in the G1 protein cytoplasmic tail. ITAMs are not present in HFRS or nonpathogenic hantavirus strains, although these strains contains a single YxxL at an identical position to that of HPS strains. Our findings demonstrate that the NY-1 hantavirus G1 ITAM interacts with Src and Syk family kinases similar to ITAMs in the cytoplasmic tails of cell surface receptors. These results demonstrate that the G1 protein cytoplasmic ITAM is functional and has the potential to alter normal intracellular signaling pathways directed by two families of protein tyrosine kinases. These findings suggest a direct role for the hantavirus G1 cytoplasmic tail in regulating immune and endothelial cell receptor signaling.

A family of immune cell receptors contain ITAM signaling elements in their cytoplasmic tails (2). These receptors convey ligand binding signals to intracellular pathways through ITAM interactions with cellular kinases. We have identified a conserved ITAM motif in the G1 protein cytoplasmic tail of all HPS-causing hantaviruses. In Fig. 1, G1 ITAM motifs from HPS- or HFRS-associated and nonpathogenic hantaviruses are presented and aligned with ITAMs from cellular and viral sources. ITAMs are not present in HFRS-causing hantaviruses, although a single YxxL is present in all hantaviruses at an identical position (Y619, Fig. 1) in the G1 cytoplasmic tail (Fig. 1). An ITAM is present in one nonpathogenic hantavirus, Tula. However, the Tula G1 ITAM is also missing an adjacent cysteine residue (amino acid [aa] 618), which is conserved in all other hantaviruses and is likely to alter the conformation and accessibility of the motif within the Tula G1. All other nonpathogenic hantaviruses lack ITAMs. The conservation of ITAMs in all HPS-associated hantaviruses sequenced to date suggests that it is functionally important to the virus and that it may contribute to HPS. The central role of ITAMs in immune cell activation responses suggests that ITAMs present in HPS-causing hantaviruses may modulate immune and endothelial cell functions by altering normal cell signaling responses.

In order to determine if hantavirus G1 ITAMs interact with cell-signaling proteins a series of experiments were performed to define ITAM interactions with cellular kinases. Phosphorylated G1 ITAM peptides synthesized by QBC, Inc. (Ac-EGCpYRTLGVFRYKSRCpYVGL-NH_2) or control peptides (NSQNSNLFISDSK-NH_2) were covalently conjugated to Affigel 102 or Affigel 10 beads (Bio-Rad), respectively, and used to precipitate protein complexes from cell lysates. Coprecipitated kinases and phosphoproteins were detected by in vitro phosphorylation in the presence of [γ-^{32}P]ATP and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (48). Phosphorylated proteins of 140, 85, 75, 59, 56, 49 and 35 kDa were precipitated with the G1 ITAM peptide, but not with a control peptide (Fig. 2). In this assay, coprecipitated proteins were detected by phosphorylation, and as a result they were either kinases that were autophosphorylated or protein substrates for a coprecipitated kinase. Based on mass, phosphoproteins coprecipitated with hantavirus G1 ITAMs are consistent with kinases involved in ITAM-directed cell signaling, including Src (52 to 60 kDa) and Syk (72 kDa) family kinases. These findings are the first to demonstrate that G1 cytoplasmic tails interact with cellular kinases and further

Hantavirus Cytoplasmic Elements		Disease
	615 638	
NY1	KQG C **YRTL** GVFR YKSR C **YVGL** VW G	HPS
SN	RK- - **Y--L** ---- ---- - **Y--L** -- -	HPS
BAY	-R- - **Y--L** ---- ---- - **Y--L** -- S	HPS
BCC	QR- - **Y--L** ---- ---- - **Y--L** -- M	HPS
ELMC	-P- - **Y--L** ---- ---- - **Y--L** -- -	?
BR	--- - **Y--L** ---- ---- - **Y--L** -- -	?
AND	-K- - **Y--L** ---- ---- - **Y--L** -- C	HPS
PRG	-R- - **Y--L** ---- ---- - **Y--L** -- C	?
LN	HR- - **Y--L** ---- -R-- - **Y--L** -- -	HPS
BMJ	RK- - **Y--L** ---- ---- - **Y--L** -- C	HPS
LEC	RK- - **Y--L** ---- ---- - **Y--L** -- C	HPS
MAC	RR- - **YK-L** ---- ---- - **Y--L** -- C	?
Hu39694	-K- - **Y--L** ---- ---- - **Y--L** -- C	HPS
ORN	-R- - **Y--L** ---- ---- - **Y--L** -- C	HPS
LSC	GK- - **Y--L** ---- ---- - **Y--L** -- C	?
CAS	RK- - **Y--L** ---- ---- - **Y--L** -- C	HPS
ARA	-R- - **Y--L** ---- ---- - **Y--L** -- C	HPS
PH	-R- - **Y--L** S--- -R-- - **F--L** -- C	None
PUU	M-- - **Y--L** SL-- -R-- F **F--L** -- C	HFRS
TUL	-R- L **Y--L** SM-- ---K - **Y--L** -- C	None
HTN	TP- - **Y--L** NL-- ---- - **YIFT** M- I	HFRS
SEO	GP- - **Y--L** NL-- ---- - **YILT** M- T	HFRS
DOB	SP- - **Y--L** NL-- ---- - **YIFT** -- V	HFRS
THAI	GP- - **Y--L** NL-- ---- - **YILT** M- L	None
ITAMS in other viruses:		
EBV-LMP2A	RHS D **YQPL** -T.Q DQ-L . **YL-L** QH -	
BLV gp30	PDS D **YQAL** .LPS .APE I **YSHL** SP V	
SIV nef	GGN L **YERL** ..LQ ARGE T **YGRL** WE -	
Receptors:		
hu CD3γ	NDQ L **YQPL** .KD- .EDD Q **YSHL** QG N	
BCR/Ig-α	DEN L **YEGL** NL.D .DCS M **YEDI**	
TCR-ζ2	QEG L **YNEL** QKDK M.AE A **YSEI** GM K	
FcERI-γ	SDA V **YTGL** N.T- NQ.E T **YEIL** KH E	
FcERI-β	DDR L **YEEL** HVY. .SPI . **YSAL** ED T	
FcyRIIA	AD- G **YMTL** NPRAPTDDDKNIYLTL PP N	
Hu PECAM-1	SDV Q **YTEVQVSSAEWSHKDLGKKDTEIVYSEVRK**	
ITAM Motif	**YxxL/I** **YxxL/I**	

FIG. 1. Amino acid comparison of ITAM elements. Amino acid alignment of the G1 protein cytoplasmic tail of hantaviruses with other viral ITAMs and cellular receptors. The G1 cytoplasmic tail ends at residue 652. Amino acid identities are indicated by dashes. ITAM motifs are shown in boldface. HPS and HFRS are diseases caused by hantaviruses and specified to the right of hantavirus strains (22, 30, 32, 39, 45). ?, HPS-associated disease has not been determined for this hantavirus. Viruses: NY, New York; SN, Sin Nombre; BAY, Bayou; BCC, Black Creek Canal; ELMC, El Moro Canyon; BR, Blue River; AND, Andes; PRG, Pergamino; LN, Laguna Negra; BMJ, Bermejo; LEC, Lechiguanas; MAC, Maciel; ORN, Oran; LSC, Limestone Canyon; CAS, Castelo dos Sonhos; ARA, Araraquara; PH, Prospect Hill; PUU, Puumala; TUL, Tula; HTN, Hantaan; SEO, Seoul; DOB, Dobrava-Belgrade; THAI, Thai-749. Other viruses: EBV-LMP2A, EBV-latent membrane protein 2A; BLV gp30, bovine leukemia virus glycoprotein. Receptors: TCR ζ2, TCR 2nd ITAM motif on zeta chain; FcεRI, high-affinity IgE receptor; FcγRIIA, low-affinity IgG receptor; PECAM-1, platelet-endothelial cell adhesion molecule-1.

suggest that ITAMs within the hantavirus G1 cytoplasmic tail are associated with cell-signaling complexes.

In order to determine if hantavirus ITAMs interact with Src family kinases, we determined whether the ITAM in the G1 cytoplasmic tail was a substrate for the Src family kinase Fyn, which is present in T cells. G1–glutathione *S*-transferase (GST) fusion proteins GST-G1-37 (contains aa 601 to 638 of NY-1 G1 protein), GST-G1-75 (aa 577 to 652 of NY-1 G1) and GST-G1-37-YY-FF (aa 601 to 638 of NY-1 G1 with tyrosines 619 and 632 mutated to phenylalanine) containing C-terminal residues of G1 were used as in vitro substrates for Fyn. Fyn kinase assays were performed by incubating 10 ng of recombi-

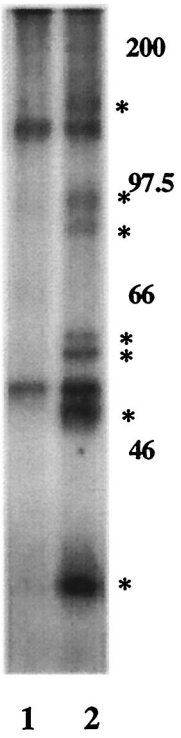

FIG. 2. Hantavirus ITAMs coprecipitate cellular kinases. Phosphorylated G1 ITAM peptides (residues 617 to 635, lane 2) or control peptides (lane 1) were covalently attached to Affigel beads and used to precipitate protein complexes from Cos-7 cell lysates. Coprecipitated kinases and phosphoproteins were detected by an in vitro phosphorylation assay in the presence of [γ-^{32}P]ATP. Phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography. Sizes are shown to the right in kilodaltons. *, phosphorylated proteins specifically coprecipitated by the ITAM peptide.

nant Fyn (Upstate Biotechnology) with GST (2 μg) or GST-fused G1 proteins (2 μg) at 37°C for 10 min in 20 μl of kinase buffer containing 5 μCi of [γ-^{32}P]ATP (48). Samples were analyzed by SDS-PAGE and visualized by autoradiography. Commercially available Fyn was capable of phosphorylating wild-type G1-ITAM-containing constructs (Fig. 3A). However, mutating ITAM tyrosines 619 and 632 to phenylalanine completely abolished Fyn phosphorylation (Fig. 3A, lane 3). The specificity of Fyn for the G1 ITAM was apparent, because an additional tyrosine present in a non-ITAM context (residue 627) was not phosphorylated by Fyn. These findings indicate that the G1-ITAM is a substrate for Src family kinases in vitro and that Fyn specificity is dependent on the presence of only ITAM tyrosines.

We further analyzed the ability of the G1 ITAM to coprecipitate the B-cell-specific Src family kinase Lyn using GST-G1 fusion proteins (Fig. 3B) (10, 44) and G1 ITAM peptides (data not shown). Figure 3B demonstrates that GST alone did not coprecipitate Lyn, while both wild-type GST-G1-75 constructs coprecipitated Lyn from Raji cell lysates. The longer GST construct, which includes what is presumed to be the native C terminus of G1, appeared to be more effective at coprecipitating Lyn. The tyrosine-mutated construct resulted in a dramatically reduced interaction with Lyn. Residual G1-Lyn interactions may be explained by some binding to an additional, non-ITAM tyrosine present at residue 627 (Fig. 3B, lane 1). These findings indicate that hantavirus G1 ITAMs coprecipitate and are substrates for Src family kinases present in B and T cells.

Once phosphorylated by Src family kinases, ITAMs are bound by paired SH2 domains within Syk/ZAP-70 family kinases. ZAP-70 is present in T cells, while Syk is present in B cells. The GST-G1-37 fusion protein coprecipitated Syk from Raji cells, whereas the tyrosine-to-phenylalanine-mutated GST-G1 failed to interact with Syk (Fig. 4A). These findings indicate that G1 interactions with Syk are dependent on the presence of tyrosines 619 and 632 within the ITAM. The G1 ITAM-containing peptide, but not the control peptide, coprecipitated the ZAP-70 kinase from the Jurkat cell line (data not shown).

We investigated cytoplasmic interactions of Syk and G1 by mammalian two-hybrid analysis. In this system, the specific interaction of two intracellularly expressed proteins are required to activate a transcription complex that directs luciferase expression. Constant amounts of ITAM/pAct, ITAM*/pAct and Syk/pBIND plasmids or empty vectors were transfected into Cos-7 cells along with the pG5luc reporter plasmid (37). Firefly and Renilla luciferase activity were measured with a dual-luciferase assay system (Promega). Samples were normalized to constant amounts of Renilla luciferase activity, which is an internal control. Neither Syk/pBIND nor ITAM/pACT constructs alone or in the presence of comple-

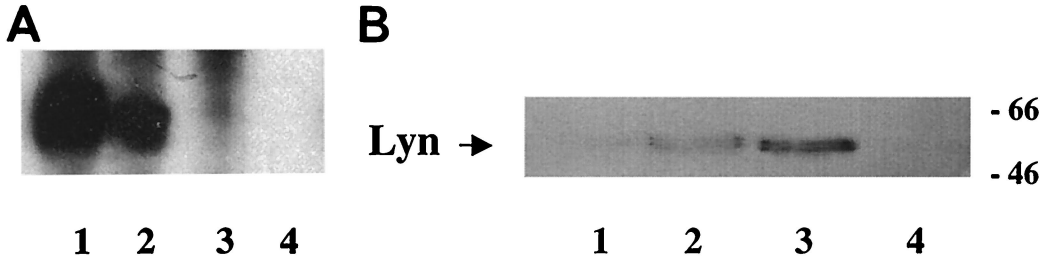

FIG. 3. G1 Interactions with Src family kinases. (A) GST-G1 fusion proteins were purified and used as substrates for in vitro phosphorylation by Fyn kinase (9). Purified recombinant Fyn was used to phosphorylate GST fused to C-terminal portions of the G1 cytoplasmic tail: GST-G1-75 (lane 1), GST-G1-37 (lane 2), GST-G1-37-YY-FF (lane 3), and GST alone (lane 4). Proteins were separated by SDS-PAGE and visualized by autoradiography. (B) GST-G1 constructs were used to coprecipitate proteins from Raji cell lysates. Lanes: 1, GST-G1-37-YY-FF; 2, GST-G1-37; 3, GST-G1-75; and 4, GST alone. Coprecipitated proteins were resolved by SDS-PAGE (10% polyacrylamide) and were Western blotted with an antibody specific for the Lyn p56/53 doublet.

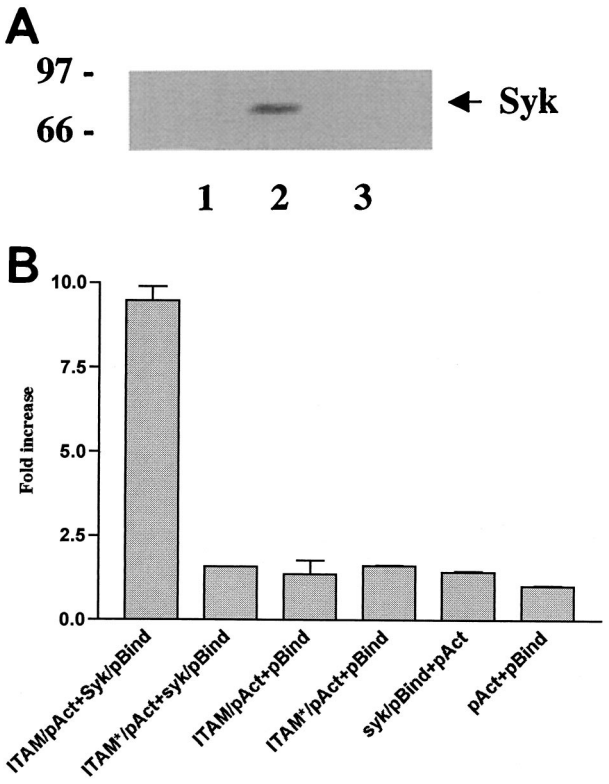

FIG. 4. Direct interactions of hantavirus G1 ITAMs with Syk. (A) GST-G1-37-YY-FF (lane 1), GST-G1-37 (lane 2), and GST alone (lane 3) were purified and used to coprecipitate Syk from Raji cell lysates. Precipitated proteins were resolved by SDS-PAGE (10% polyacrylamide) and immunoblotted with antibody to Syk. (B) Mammalian two-hybrid analysis of G1 ITAM-Syk interactions. Cos7 cells were transfected with a constant amount (5 μg each) of ITAM/pAct (612 to 652 aa of NY-1 G1 protein fused to VP16), ITAM*/pAct (same as the ITAM/pAct construct with tyrosines 619 and 632 mutated to phenylalanines), Syk/pBind (1 to 634 aa of full-length Syk fused to Gal4), or control plasmids (pAct or pBind) along with a pG5luc luciferase reporter. Lysates were harvested 36 h posttransfection and lysed in Promega passive lysis buffer. Firefly and Renilla luciferase activities were measured with a dual-luciferase assay system. Renilla luciferase was used as an internal control for normalization. The data are presented as the fold luciferase induction. Assays were performed at least three times with similar results.

mentary empty vectors activated luciferase reporters. However, coexpression of both Syk and ITAM/pACT specifically directed transcription from the luciferase reporter (Fig. 4B). This demonstrated that Syk and G1 formed specific cytoplasmic complexes within cells and the ITAM*/pACT mutant failed to interact with Syk, indicating that G1 ITAM tyrosines are required for Syk binding to the G1 cytoplasmic tail. Collectively, these findings indicate that the hantavirus G1 ITAMs bind Src and Syk family tyrosine kinases and suggest a means for HPS causing hantaviruses to alter normal immunoreceptor cell signaling.

Hantaviruses predominantly infect endothelial cells and are reported to infect a number of immune cells (34, 36, 40, 54, 61, 63). However, there is little information on hantavirus infection of immune cells and no information on how hantaviruses direct or regulate endothelial or immune cell responses (34, 50, 54, 62, 63). Hantaviruses cause two types of vascular permeabilty-related diseases, HPS and HFRS (25, 36, 54, 63). Although cytokines and integrins have been forwarded as contributers to hantavirus pathogenesis, there is little understanding of how hantaviruses cause disease (13–15, 28, 33, 40, 49, 50, 54). It is likely that hantavirus pathogenesis is multifactorial, involving a combination of effects that include chemokines, cytokines, cell surface receptors, immune cell recruitment, endothelial cell alterations, and viral regulation of these processes. Altering cell signaling provides one means for hantaviruses to regulate cellular responses and contribute to pathogenesis.

We have found that all HPS-causing hantaviruses contain a conserved ITAM signaling motif within their G1 cytoplasmic tail. We have demonstrated that the hantavirus G1 ITAM binds cellular Src and Syk family kinases that normally direct immune and endothelial cell signaling responses. The G1 ITAM coprecipitates a combination of at least five proteins that are either kinases themselves or substrates for coprecipitated kinases, suggesting that signaling complexes are recruited to the G1 cytoplasmic tail. As a result, we have identified a signaling element within hantaviruses that has the capacity to regulate cellular transcriptional responses, as well as immune and endothelial cell functions, and contribute to the vascular permeability deficits that are hallmarks of hantavirus diseases.

Several viruses have been shown to alter immune cell function by introducing ITAMs into infected cells and thereby modulating normal ITAM-directed immune cell signaling (1, 8, 11, 29, 31). The EBV LMP2A protein contains an ITAM that blocks BCR-directed signaling (11, 29). Similar to what we have shown here for hantavirus ITAMs, LMP2A ITAMs bind Src and Syk kinases and thereby down regulate the function of Src, Syk, and ITAM-containing receptors within infected immune cells (11, 31).

The fact that the first YxxL is present in the G1 cytoplasmic tail of all hantaviruses also suggests a requirement for this signaling motif in regulating common cell functions or cellular responses. In bovine leukemia virus, a YxxL is required for in vivo infection and maintenance of high viral loads, although it is unknown how the YxxL contributes to this phenotype (17, 58). Thus, single YxxL elements could contribute to viral assembly or persistence in animal hosts or function as ITIMs that regulate immune responses that would clear the virus. Since all hantaviruses contain at least one YxxL, a single amino acid change could introduce an ITAM element into the G1 protein. The effect could be a change from an ITIM inhibitory response to an ITAM activation response within cells and thereby contribute to specific HPS-associated effects within endothelial cells. If so, HPS-causing hantaviruses could have emerged from nonpathogenic or HFRS-causing hantaviruses by introducing an ITAM into G1. Unfortunately, reverse genetics have not been established for hantaviruses, and it may be some time before roles for ITAMs, immunoreceptor tyrosine-based inhibition motifs (ITIMs), or YxxLs in hantavirus pathogenesis, persistence, or immune cell function can be conclusively established.

HPS-causing hantaviruses infect endothelial cells and could also regulate ITAM functions within them. Only recently has a role for receptor ITAMs in endothelial cell function been presented. Platelet-endothelial cell adhesion molecule (PECAM-1, CD31) contains tyrosines (Y^{663} and Y^{686}) that con-

form to the consensus sequences for ITAMs (Fig. 1) (26). PECAM-1 is a 130-kDa glycoprotein belonging to the Ig superfamily of cell adhesion molecules and PECAM-1 expression is restricted to cells of the vascular system including endothelial cells infected by hantaviruses (35). PECAM-1 ITAMs undergo phosphorylation following mechanical or biochemical stimulation and PECAM-1 is phosphorylated by c-Src (26). PECAM-1 associates with SHIP, an inositol phosphatase that, like Syk and ZAP-70, binds ITAMs. (41). In fact, PECAM-1 tyrosines are also consistent with ITIMs, which down regulate ITAM receptor signaling (16, 21, 26, 41). PECAM-1 binds SHP-1 and SHP-2 phosphatases, and as a result, PECAM-1 may function both as an ITAM- and an ITIM-bearing receptor in directing endothelial cell signaling responses. Since the role of ITAM signaling in endothelial cells is a newly emerging field, more roles for endothelial cell ITAMs are likely to be discovered that may shed light on how hantavirus ITAMs may contribute to pathogenesis.

Interestingly, Syk signaling is also tied to endothelial cell dysfunction and vascular permeability defects, since Syk-deficient mice die in utero from microvascular hemorrhage (5, 52). Hemorrhage in Syk-deficient mice appears to be caused by abnormal morphogenesis of vascular endothelial cells indicating that Syk is required for normal endothelial cell function (60). It has also been shown that overexpression of a dominant-negative Syk suppresses normal signaling pathway activation in endothelial cells (18). These and other studies have shown that Syk plays a critical role in endothelial cell morphogenesis, growth, migration, cell survival, and contributes to maintaining vascular integrity in vivo (5, 18, 51–53, 60). As a result, hantavirus G1 ITAM-Syk interactions could modulate endothelial cell functions that maintain vascular integrity and specifically contribute to HPS.

The presence of Src- and Syk-interactive ITAM elements within HPS-causing hantaviruses and their link to vascular permeability effects suggest that G1 ITAMs could contribute to hantavirus pathogenesis by regulating endothelial cell functions. Alternatively, the enhanced pathogenesis of SIV strains containing Nef-ITAMs suggest that hantavirus ITAMs could represent a virulence factor that manifests its effects on immune cells (8). In either case, these findings suggest that a hantavirus signaling element linked to viral virulence may represent a specific determinant of HPS disease.

We thank Joanne Mackow for technical assistance.

This work was supported by a Merit Award from the Veterans Administration and by NIH AI047873 and AI044917.

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