Cellular Receptors and Hantavirus Pathogenesis

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1 Introduction

Hantaviruses cause two frequently fatal human diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) (Nolte et al. 1995; Zaki et al. 1995; Gonzalez-Scarano et al. 1996; Schmaljohn 1996). HFRS-associated hantaviruses cause vascular hemorrhage and kidney dysfunction while HPS-associated hantaviruses cause acute pulmonary edema (Lee et al. 1999; Zaki et al. 1999). Both diseases are associated with acute thrombocytopenia and changes in vascular permeability, and either disease may have pulmonary or renal components (Nolte et al. 1995; Zaki et al. 1995; Gonzalez-Scarano and Nathanson 1996; Schmaljohn 1996; Lee et al. 1999; Zaki and Nolte 1999).

The means by which specific hantaviruses cause pulmonary or renal diseases and increase vascular permeability is just beginning to be investigated. Hantaviruses cause disease in humans but not in their animal hosts, and both pathogenic and non-pathogenic hantaviruses have the same tissue tropism, replicating predominantly in endothelial cells and macrophages (Yanagihara and Silverman 1990; Pensiero et al. 1992; Hughes et al. 1993; Nolte et al. 1995; Zaki et al. 1995; Gonzalez-Scarano and Nathanson 1996). Infected endothelial cells are not lysed by hantaviruses, and although immune cells are recruited to the infected endothelium (Zaki et al. 1995), it is unclear to what extent these contribute to vascular disease (Yanagihara and Silverman 1990; Nolte et al. 1995). Recent findings indicate that pathogenic and non-pathogenic hantaviruses interact with discrete integrin receptors present on platelets, immune and endothelial cells (Gavrilovskaya et al. 1998; Gavrilovskaya et al. 1999; Mackow et al. 1999). These findings link hantavirus pathogenesis to the use of β3 integrins, which are central to the regulation of platelet function and vascular permeability (Gavrilovskaya et al. 1998; Gavrilovskaya et al. 1999; Mackow et al. 1999).

2 Hemorrhagic Fever with Renal Syndrome

HFRS was first described over 50 years ago, and hantaviruses were first isolated in 1978 (Lee et al. 1978; Schmaljohn et al. 1997). Hantaviruses derive their name from the Hantaan river in Korea where Hantaan virus, the prototype HFRS-causing hantavirus, was found to be the etiologic agent of Korean hemorrhagic fever (Lee et al. 1978). Hantaan (HTN), Seoul (SEO), Puumala (PUU) and Dobrava (DOB) viruses are prominent causes of human HFRS and induce various degrees of disease with 0.1–5% mortality rates (Lee 1982; Cosgriff et al. 1991; Avsic-Zupanc et al. 1992). HFRS disease is characterized by an influenza-like illness with microvascular hemorrhage, platelet loss, hypotension, shock, and in some cases renal failure (Lee 1982; Cosgriff and Lewis 1991; Avsic-Zupanc et al. 1992). Activation of complement cascades in HFRS patients supports a role for

accumulated immune complexes in renal disease (GAVRILOVSKAYA et al. 1987). However, the mechanism by which HFRS viruses cause vascular hemorrhage, thrombocytopenia, and kidney disease are not well understood.

Hantaan virus is estimated to cause over 100,000 cases of HFRS annually in Asia. Seoul virus is present worldwide, although reported cases occur predominantly within Asia. Puumala is a substantial cause of HFRS morbidity within Europe, and Dobrava virus is a newly identified cause of severe HFRS within the Balkan region of Europe. Although PUU infections are characterized by a low mortality rate, this and other hantaviruses are severely debilitating and require prolonged recovery periods lasting from months to more than a year. A more complete description of hantavirus clinical disease and epidemiology can be found in several reviews (GONZALEZ-SCARANO and NATHANSON 1996; SCHMALJOHN 1996; SCHMALJOHN and HJELLE 1997; LEE et al. 1999; ZAKI and NOLTE 1999).

3 Hantavirus Pulmonary Syndrome

In 1993, adult respiratory distress (ARDS) cases in the southwestern United States led to the discovery of Sin Nombre virus (SNV) as the etiologic agent of Hantavirus Pulmonary Syndrome (HPS), a new acute respiratory disease (HJELLE et al. 1994; NOLTE et al. 1995; ZAKI et al. 1995; ZAKI and NOLTE 1999). Since then, HPS-associated viruses have been identified in at least 28 US States, Canada, and South America. With a 40–45% mortality rate, HPS infections are highly lethal to infected patients. Influenza-like symptoms progress rapidly to acute respiratory distress resulting from bilateral infiltrates within the patients lungs (NOLTE et al. 1995; ZAKI et al. 1995). The main clinical manifestations of HPS cases are increased vascular permeability, interstitial pneumonia with mononuclear cell infiltrates, severe edema of lung tissue, and acute thrombocytopenia (NOLTE et al. 1995; ZAKI et al. 1995; ZAKI and NOLTE 1999). Patients succumb to increased pulmonary edema, which causes pulmonary insufficiency (NOLTE et al. 1995; ZAKI et al. 1995).

4 Non-Pathogenic Hantaviruses

Although the majority of hantaviruses found to date cause human disease, there are a few hantaviruses which are not associated with pathogenesis. Prospect Hill (PH), Tula (TUL), Thai 749, and Thottapalayam (TPM) have not been associated with any human disease (SCHMALJOHN and HJELLE 1997). PH virus was first identified in the eastern United States 11 years before the 1993 HPS outbreak in the west, but it appears to be present throughout North America (YANAGIHARA et al. 1987). There is one report that experimental infection with PH caused a mild transient renal

syndrome in lower primates, although no associated disease has been reported in humans (YANAGIHARA et al. 1988). Tula is present in Europe, and Thai and Thottapalayam are present in southeast Asia, but they have not been studied extensively (SCHMALJOHN and HJELLE 1997).

5 Common Elements of Hantavirus Disease

Both HFRS and HPS diseases have common elements. Both diseases result in increased vascular permeability and acute thrombocytopenia, although either disease may have pulmonary or renal components. Pathogenic and non-pathogenic hantaviruses all replicate predominantly within pulmonary endothelial cells. However, there is no apparent damage to endothelial cells of humans nor animals (NOLTE et al. 1995; ZAKI et al. 1995; LEE et al. 1999). Human hantavirus infections are zoonotic, although a report from South America suggests that a case of person-to-person transmission may have occurred with the HPS-associated Andes hantavirus (LOPEZ et al. 1996; LOPEZ et al. 1997). However, there have been no prior or additional reports of person-to-person transmission of HFRS- or HPS-causing hantaviruses.

6 Virus–Host Interactions

Hantaviruses are transmitted to humans from small mammal hosts through inhalation of excreted virus (LEE et al. 1978; SCHMALJOHN 1996). There are a variety of small mammals which serve as hosts for hantaviruses. However, viruses and hosts appear to have co-evolved and specific hantaviruses are primarily associated with a singular species. As a result, the range of the animal host defines the geographic range of the hantavirus and delimits human HFRS or HPS diseases from specific hantaviruses.

- HFRS-causing hantaviruses: Hantaan virus (HTN) is carried by the field mouse, *Apodemus agrarius*, in Asia. Dobrava virus (DOB) is also carried by a discrete species of *Apodemus* (*A. flavicolus*) in the Balkans, Puumala virus (PUU) is carried by bank voles (*C. glariolus*) in Europe, and Seoul virus (SEO) is carried by rats (*R. norvegicus*) worldwide (SCHMALJOHN and HJELLE 1997).
- HPS-causing hantaviruses: SNV is carried by the deer mouse (*P. maniculatus*), and NY-1 by the white-footed mouse (*P. leucopus*) in North America (NICHOL et al. 1993; HJELLE et al. 1995). Many additional genetically and antigenetically distinct hantaviruses are now associated with human HPS in North and South America and are derived from hosts unique to the area of the infection. A complete description of additional HPS hosts and host range can be found in

several articles (NICHOL et al. 1993; ELLIOTT et al. 1994; HJELLE et al. 1994; ROLLIN et al. 1994; HJELLE et al. 1995; LI et al. 1995; MACKOW et al. 1995; RAVKOV et al. 1995; SCHMALJOHN et al. 1995; LOPEZ et al. 1996; LOPEZ et al. 1997; SCHMALJOHN and HJELLE 1997; GAVRILOVSKAYA et al. 1999).

- Non-pathogenic hantaviruses: PHV and Tula viruses are carried by species of *Microtus* (*M. pennsylvanicus* and *M. arvalis*) and are respectively present in the United States and Europe. Thai 749 and Thottapalayam are carried by *Bandicota indica* and *Suncus murinus*, respectively (YANAGIHARA et al. 1987; ARTHUR et al. 1992; CHU et al. 1994).

In both animals and man hantavirus replication occurs predominantly in pulmonary endothelial cells and macrophages, although viral antigen is present in many organs and prominent in the spleen and kidney as well as the lung (YANAGIHARA and SILVERMAN 1990; PENSIERO et al. 1992; HUGHES et al. 1993; NOLTE et al. 1995; ZAKI et al. 1995; GONZALEZ-SCARANO and NATHANSON 1996; SCHMALJOHN 1996). However, hantavirus infection of animals does not adversely affect or shorten the host's life span (GAVRILOVSKAYA et al. 1990; SCHMALJOHN and HJELLE 1997). Hantaviruses are highly viremic for the first month after infection (GAVRILOVSKAYA et al. 1990; HUTCHINSON et al. 2000). However, animals are persistently infected and still capable of transmitting the virus for over a year. Although serum antibodies appear about 10 days post-infection and result in high-level neutralizing antibody responses, hantaviruses are not cleared from their rodent hosts (CHU et al. 1994). Patients similarly develop neutralizing antibody responses to the virus but are able to clear the virus, suggesting that hantaviruses regulate rodent cellular host responses to effect viral persistence.

Although hantaviruses are carried by a variety of "mice", laboratory mice (*Mus musculus*) are not hantavirus hosts. As an example of the animals that are often termed "mice" that carry hantaviruses, HPS-associated SNV and NY-1 hantaviruses infect *Peromyscus* species, which are more closely related to hamsters or voles than they are to laboratory mice or rats. Although there are varied reports on hantavirus infection of newborn lab mice, there are no reports that *Mus musculus* is a hantavirus host. Newborn lab mice injected with HTN virus reportedly develop a fatal encephalitis and infection of the endothelium (KIM and MCKEE 1985; MCKEE et al. 1985; ASADA et al. 1987; TAMURA et al. 1989). However, 3-week-old mice inoculated with HTN virus develop neither disease nor persistence (KIM and MCKEE 1985; ASADA et al. 1987; TAMURA et al. 1989). Exposure of lab mice to highly infected *A. agrarius* hosts carrying HTN also failed to transmit the virus to *M. musculus* (KIM and MCKEE 1985; MCKEE et al. 1985; GONZALEZ-SCARANO and NATHANSON 1996). Although there is less known about the transmission of HPS-causing hantaviruses, it is clear that *Mus musculus* are not hosts for HPS-causing hantaviruses.

The lack of disease in hantavirus hosts and the lack of an animal model of hantavirus disease has limited our understanding of hantavirus pathogenesis. The absence of cell lines from host species has also prevented a basic analysis of cellular differences that might contribute to pathogenic and non-pathogenic viral pheno-

types. However, the ability of hantaviruses to infect human endothelial cells is a common feature, which permits a focus on hantavirus endothelial cell interactions that may contribute to viral pathogenesis.

7 Cellular Interactions: Keys to Understanding Hantavirus Pathogenesis

Viral attachment and entry into cells is often a major determinant of cellular susceptibility to viral infections. Cellular receptors dictate tissue and cell tropism of viruses and can facilitate viral virulence or host-range restrictions for viral infections (REN et al. 1990; NORKIN 1995). Viral attachment to cells may also stimulate a cascade of intracellular signal transduction events that may activate or regulate cells with pathologic consequences.

The means by which specific hantaviruses cause pulmonary or renal diseases is obscure. Although determinants of pathogenesis have not been defined for any hantavirus, endothelial cells and platelets are prominent regulators of vascular function, and integrins play key roles in barrier functions of these cells (LAMPUGNANI et al. 1991; HAWIGER 1995; TSUKADA et al. 1995; MOGFORD et al. 1996; WU et al. 1998; HODIVALA-DILKE et al. 1999; HYNES et al. 1999; LOSKUTOFF et al. 1999). All hantaviruses infect and replicate in endothelial cells, suggesting that a simple difference in tissue tropism is not a likely determinant of pathogenic or non-pathogenic hantavirus phenotypes (YANAGIHARA and SILVERMAN 1990). This is further suggested by the identical tissue tropism of pathogenic and non-pathogenic hantaviruses within their natural hosts and the lack of disease within animals. However, little is known about hantavirus infection of endothelial cells and immune cells, and their interactions with platelets. These hantavirus cell interactions are likely to be keys to our understanding of: (a) why hantaviruses cause vascular disease; (b) why hantaviruses cause disease in humans but not their animal hosts; (c) why hantaviruses are not cleared from their hosts; (d) what determines host susceptibility; and (e) how hantaviruses establish persistent host infections.

7.1 Endothelial Cell Interactions

All hantaviruses primarily infect pulmonary endothelial cells, although viral antigen is found in endothelial cells in a variety of tissues as well as in immune cells. Our knowledge of hantavirus infections of humans are primarily based on the analysis of pathology specimens and tissue culture interactions with endothelial cells (primarily human umbilical vein endothelial cells, HUVECs) (YANAGIHARA and SILVERMAN 1990; NOLTE et al. 1995; ZAKI et al. 1995; ZAKI and NOLTE 1999). Endothelial cells and platelets regulate vascular permeability by initiating or regulating clotting and complement cascades, or by engaging in a

variety of cell–cell interactions which regulate platelet and immune cell recruitment to the vascular endothelium (CHERESH 1987; JACONI et al. 1991; HYNES 1992; LEAVESLEY et al. 1993; YLANNE et al. 1993; HEMLER et al. 1994; LOFTUS et al. 1994; SHATTIL et al. 1994; CHANG et al. 1995; SALCEDO and PATARROYO 1995; HALVORSON et al. 1996; SUEHIRO et al. 1996; SEIFFERT et al. 1997; SUGIMORI et al. 1997; HODIVALA-DILKE et al. 1999; HYNES et al. 1999; LOSKUTOFF et al. 1999). Several lines of evidence presented below lead to the understanding that cellular integrins are central players in hantavirus–endothelial cell interactions and the differential use of β3 and β1 integrins by pathogenic and non-pathogenic hantaviruses link integrin usage to hantavirus-induced vascular disease (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999).

Integrins are heterodimeric receptors composed of a combination of α and β subunits that mediate cell–cell adhesion, platelet aggregation, Ca^{+2} channel activation and extracellular matrix (ECM) protein recognition (CHERESH 1987; PHILLIPS et al. 1988; BOSSU et al. 1989; LAMPUGNANI et al. 1991; SEPP et al. 1994; HAWIGER 1995; MOGFORD et al. 1996; MOGFORD et al. 1997; SUGIMORI et al. 1997; WU et al. 1998; HYNES et al. 1999). Integrin–ligand interactions mediate the activation and regulation of intracellular signaling pathways that further control both transcriptional and ligand binding functions (CLARK et al. 1994; SHATTIL et al. 1994; SCHWARTZ et al. 1995; FAULL et al. 1996). Integrins are also linked to intracellular cytoskeletal elements that facilitate cellular migration on the ECM. ECM proteins such as vitronectin and fibronectin contain Arg-Gly-Asp (RGD) tripeptides, which are recognized by specific cellular integrins including $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$, and $\alpha_5\beta_1$ (SUGIMORI et al. 1997). $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins are abundant surface receptors of endothelial cells and platelets, respectively, and $\alpha_v\beta_3$ integrins are present on macrophages (CHERESH 1987; PHILLIPS et al. 1988; SEPP et al. 1994; HYNES et al. 1999; HYNES and HODIVALA-DILKE 1999).

7.2 Hantavirus Entry

The mechanism of hantavirus entry into cells has been addressed using inhibitors of endocytosis, lysosomotropic agents, bafilomycin A, and concanamycin (MACKOW et al. 1999). Lysosomotropic agents inhibit entry of hantaviruses by 70–80% compared to controls or rhesus rotavirus (RRV), which enters by direct membrane penetration (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). Endosome-specific H^+/ATPase inhibitors bafilomycin A and concanamycin (Folimycin) completely block hantavirus entry into cells, demonstrating that endosome acidification is required for viral entry. These findings are consistent with the sensitivity of hantavirus to pH 6 treatment and findings that indicate G1:G2 heterodimers are not formed under these conditions (unpublished observations). Although hantavirus and cell-membrane interactions have not been studied, these results suggest that endosome acidification changes the conformation of G1:G2 heterodimers and permits viral and cellular membrane fusion effecting hantavirus entry.

7.3 Hantavirus Cell Interactions

Initially hantavirus interactions with cell surface components were studied by pretreating cells with enzymes that might abolish viral infectivity. Neuraminidase pretreatment of cells had no effect on hantavirus infectivity, suggesting that hantavirus cell interactions are sialic acid independent (GAVRILOVSKAYA et al. 1998). In contrast, protease pretreatment of cells effectively inhibited hantavirus infectivity, demonstrating that hantaviruses enter endothelial cells by interacting with a proteinaceous cell surface receptor that is internalized through acidified endosomes (GAVRILOVSKAYA et al. 1998; MACKOW et al. 1999).

8 Specific Integrins Mediate Hantavirus Entry into Cells

Cellular receptors for the NY-1 hantavirus were studied by pretreating cells with ligands or antibodies to potential receptors and subsequently analyzing viral entry by immunostaining viral-infected cells 36h post-infection (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). A synopsis of the data, which indicate that β_3 integrins mediate the infection of HPS and HFRS-associated hantaviruses and β_1 integrins mediate the entry of the non-pathogenic PH and Tula hantaviruses, is presented in the following sections (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999).

8.1 Integrin Ligands Block Hantavirus Infection

In order to determine whether integrins or additional cell surface receptors mediate the cellular entry of HPS- and HFRS-causing hantaviruses, the ability of ligands to inhibit infection by NY-1, SN, HTN, SEO, PUU, and PH viruses was assessed. A number of ligands to cell surface receptors were tested for their ability to block hantavirus infectivity. Potentially competitive inhibitor proteins, carbohydrates, and lectins were preadsorbed to cells, removed, and cells were then infected to determine the ability of prebound ligands to block virus infectivity. Of the compounds tested, only two ligands had an effect on hantavirus infectivity, vitronectin and fibronectin, and these ligands selectively inhibited pathogenic and non-pathogenic hantaviruses, respectively (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). Pretreatment of cells with vitronectin inhibited the infectivity of pathogenic NY-1, SNV, HTN, SEO, and PUU viruses (>70%) (Fig. 1) (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). In contrast, non-pathogenic PHV, Tula, and TPM viruses were not inhibited by vitronectin but were blocked by fibronectin (Fig. 1). These results suggest that a vitronectin binding cell surface protein serves as a receptor for pathogenic hantaviruses.

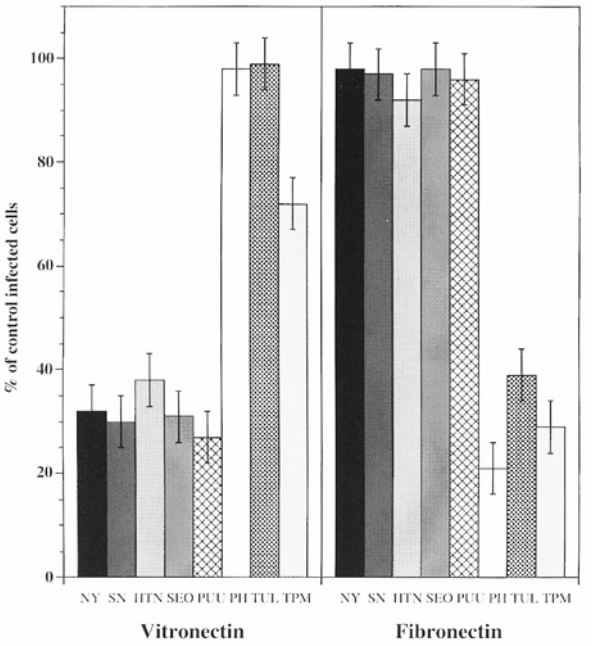

Fig. 1. Ligand-specific inhibition of hantavirus infectivity. Potentially competitive ligands (5–40μg/ml, 50μl) were preadsorbed to Vero E6 cells for 1h before viral adsorption. Approximately 400 FFUs of NY-1, SN, HTN, SEO, PUU, PH, TUL, and TPM hantaviruses were adsorbed to duplicate wells of a 96-well plate. Following adsorption, inocula were removed, and cells were washed and further incubated 24–36h at 37°C in 5% CO_2 before methanol fixation. Hantavirus-infected cells were immunoperoxidase stained as previously described (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999) using polyclonal rabbit anti-nucleocapsid sera made to bacterially expressed and nickel affinity purified NY-1 N-protein. Infected cells were quantitated and compared to control infections without competitor proteins. Results were reproduced in at least three separate experiments. Results are presented as the percent inhibition of control infections. Pretreatment with 1–500μg/ml BSA did not affect hantavirus infectivity

8.2 Integrin-Specific Antibodies Inhibit Pathogenic and Non-pathogenic Hantaviruses

The ability of the integrin-specific ligands vitronectin and fibronectin to inhibit hantavirus infectivity suggests that integrins facilitate hantavirus entry into cells. In order to directly test this, integrin-specific antibodies, as well as antibodies to other cellular receptors, were tested for their ability to inhibit hantavirus infectivity. Antibodies were prebound to HUVECs or Vero E6 cells. Subsequently, 200 FFUs (focus-forming units) of each hantavirus was adsorbed to cells and infected cells were quantitated 24–36h post-infection. Antibodies to only $\alpha_v\beta_3$ and β_3 were capable of inhibiting infection by pathogenic hantaviruses (SNV, NY-1, HTN, SEO, PUU) (Fig. 2). In contrast, antibodies to additional integrin subunits or other endothelial cell surface proteins did not inhibit infection by pathogenic hantaviruses. Non-pathogenic hantaviruses PHV, Tula, and TPM were not blocked by α_v- or β_3-specific sera and instead were specifically inhibited by sera to α_5 and β_1

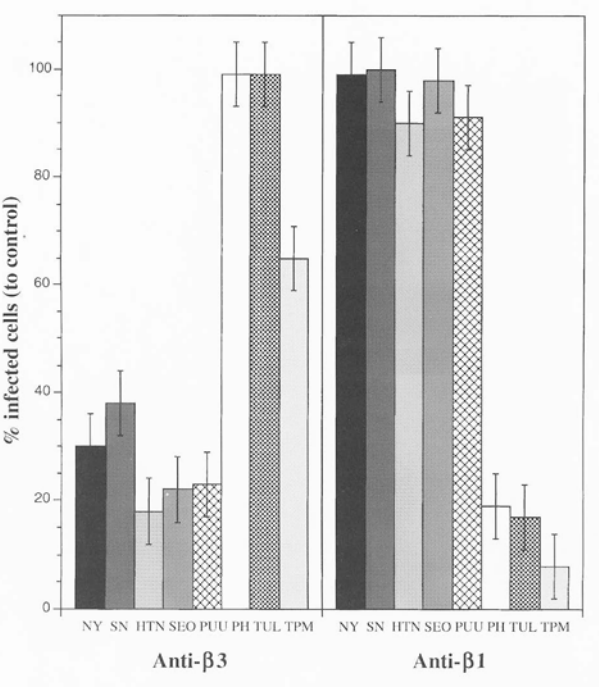

Fig. 2. Infectivity of hantaviruses by integrin-specific antibodies. Duplicate wells of Vero E6 cells were pretreated for 1h (37°C) with 20μg/ml of antibodies to specific integrins. Monolayers were washed and NY-1, SN, HTN, SEO, PUU, PH, TUL, and TPM hantaviruses were adsorbed (Fig. 1) (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). Infected cells were quantitated as in Fig. 1. FFUs observed 36h post-infection are expressed as a percent of control infections for each viral inoculum. Polyclonal rabbit sera to β_1, β_3 were from Chemicon

integrins. $\alpha_v\beta_3$-specific sera inhibited NY-1, SNV, HTN, SEO, and PUU infectivity by approximately 70–90% while failing to block PH infectivity (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). Reciprocally, antisera to $\alpha_5\beta_1$ blocked PH, Tula, and TPM infectivity (>80%) but failed to inhibit NY-1, SNV, HTN, SEO or PUU infections (Fig. 2). These results suggested that β_3-specific integrins mediate the cellular entry of pathogenic hantaviruses, which cause HPS and HFRS. The use of unique integrins by non-pathogenic hantaviruses further suggested that hantavirus pathogenesis may be linked to discrete integrin usage.

8.3 Recombinant Integrins Confer Cell Susceptibility to Pathogenic Hantaviruses

CHO cells lack β_3 integrins and are not permissive for hantavirus infection. In order to demonstrate that β_3 integrins are cellular receptors for pathogenic hantaviruses, we transfected CHO or HEK cells with human recombinant $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ integrins (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). Cell lines

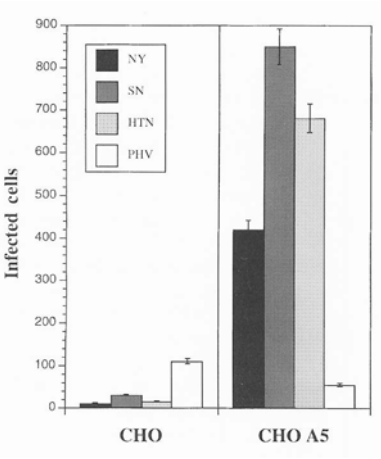

Fig. 3. Recombinant β_3 integrins render CHO cells permissive pathogenic hantaviruses. CHO cells transfected with the $\alpha_{IIb}\beta_3$ integrin (CHO-A5 cells) and infected with NY-1, SN, HTN, and PHV. Following immunoperoxidase staining of nucleocapsid protein in cells, infected cells were quantitated as in Fig. 1 (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999)

were tested for their susceptibility to hantavirus infection by quantitating infected cells 24–36h post-infection. CHO or HEK cells containing $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ (CHO A5 cells) integrins were susceptible to SNV, NY-1, HTN, SEO, and PUU virus infections but not to non-pathogenic PHV or Tula hantaviruses (Fig. 3). In addition, pretreatment of CHO cell lines containing recombinant β_3 integrins with β_3 integrin-specific sera inhibited infection by pathogenic hantaviruses. These findings demonstrate that human $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins confer cellular susceptibility to HPS- or HFRS-causing hantaviruses.

8.4 Co-receptors

Regardless of the integrin ligand or antibody blocker employed, some cells are still infected by hantaviruses. Even though some anti-β_3-specific sera block infectivity >95%, the possibility of a hantavirus co-receptor on cells cannot be ruled out. One candidate for a hantavirus co-receptor is the 50-kDa integrin-associated protein (IAP), which is complexed with cellular β_3 integrins. However, IAP-specific antibodies failed to block hantavirus infectivity or to enhance the inhibitory effects of β_3 integrin-specific sera (Fig. 4) (GAVRILOVSKAYA et al. 1999). As a result, it is unlikely that IAPs facilitate hantavirus entry or are required to be complexed with β_3 integrins for entry.

8.5 Human Monoclonal Antibodies to β_3 Integrins Block Hantavirus Infectivity

Humanized antibodies to β_3 integrins are used therapeutically as anti-thrombotic agents. c7E3 (ReoPro) is a commercially available $\alpha_v\beta_3$-specific mouse–human hybrid Fab fragment that is used clinically to inhibit thrombus formation in and around vascular stints (REVERTER et al. 1996; COLLER 1997a,b). Pretreatment of

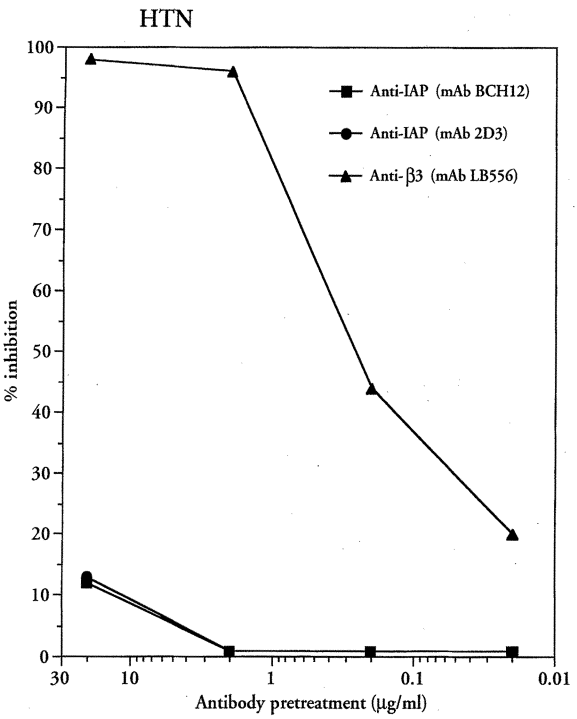

Fig. 4. HTN infectivity is not inhibited antibodies to integrin-associated protein. Duplicate wells of Vero E6 cells were pre-treated with 20ng/ml to 20μg/ml monoclonal antibodies, 2D3, and B6H12, to the 50-kDa integrin-associated protein for 1h at 37°C or with mAb LB556, which is specific for β3 integrin subunits (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). Monolayers were washed three times with PBS and infected with approximately 400 FFUs of HTN virus. Infected cells were quantitated as in Fig. 1 and results are presented as the percent inhibition of a control infection

cells with c7E3 or Vitaxin (a distinct hybrid antibody to β_3) reduces the infectivity of SNV, NY-1, HTN, SEO, and PUU viruses by 50–80% while having no effect on the infectivity of PHV or Tula viruses (Fig. 5) (GAVRILOVSKAYA et al. 1999). These findings suggest that anti-β_3 integrin antibodies may be useful therapeutic reagents against hantavirus-induced disease. However, the lack of an animal model of hantavirus disease limits the ability to test the effect of these and other reagents on hantavirus disease processes.

8.6 Hantavirus Interaction with Integrins Is RGD Independent

β_3 subunits confer cell susceptibility to pathogenic hantaviruses, and $\alpha_v\beta_3$ integrins appear to be receptors for pathogenic hantaviruses on endothelial cells. Many integrin ligands bind integrin receptors through RGD tri-peptides, although the specificity of the integrin ligand binding interactions is also mediated by additional, selective interactions (CHERESH 1987; LOFTUS et al. 1990; HUTTENLOCHER et al. 1996; SUEHIRO et al. 1996; TOZER et al. 1996; SUGIMORI et al. 1997; HYNES et al. 1999). However, hantaviruses lack RGD motifs in their G1 and G2 surface glycoproteins, which precludes RGD-specific hantavirus interactions with integrins.

Divalent cations also regulate the high affinity binding state of integrins. Manganese addition enhances high affinity RGD binding by integrins while calcium ion addition inhibits RGD-integrin interactions (SUGIMORI et al. 1997).

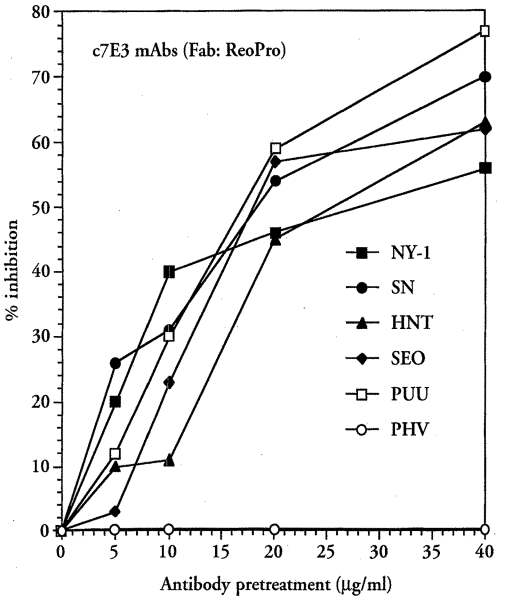

Fig. 5. Fab c7E3 (ReoPro) inhibits the infectivity of HPS- and HFRS-causing hantaviruses. Duplicate wells of Vero E6 cells were pretreated with 20ng/ml to 40µg/ml mouse–human hybrid Fab, c7E3, to $\alpha_v\beta_3$ for 1h at 37°C. Monolayers were washed three times with PBS and infected with approximately 400 FFUs of HTN, SEO, PUU, NY-1, SN, and PH viruses. Infected cells were quantitated as in Fig. 1, and results are presented as the percent inhibition of control infections (GAVRILOVSKAYA et al. 1998, 1999)

Consistent with an RGD independent interaction of hantaviruses with integrins, pretreatment of cells with Mn^{+2} blocks hantavirus infectivity while Ca^{+2} enhances hantavirus infection of cells (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). These results further support the RGD independence of hantavirus integrin interactions and may explain why RGD containing ligands, such as fibronectin, do not block the infectivity of pathogenic hantaviruses (GAVRILOVSKAYA et al. 1998). The lack of an effect of fibronectin on pathogenic hantaviruses may also be explained by the fact that fibronectin is not a high affinity ligand for β_3 integrins (SUGIMORI et al. 1997).

In order to test the RGD independence of hantavirus integrin interactions, experiments were performed with RGD peptides and β_3 integrins, which lack the ability to bind RGD ligands. RGD and RGE peptides were added as competitive inhibitors to determine their ability to block the infection of pathogenic or non-pathogenic hantaviruses (GAVRILOVSKAYA et al. 1998, 1999; MACKOW et al. 1999). At concentrations as high as 200µg/ml, RGD peptides failed to inhibit the infection of any hantavirus tested (NY-1, SNV, HTN, PUU, SEO, PHV, Tula) (GAVRILOVSKAYA et al. 1998, 1999). When a mutant β_3 integrin, which lacks the ability to bind RGD ligands, was transfected into cells, pathogenic hantaviruses were still able to infect cells (GAVRILOVSKAYA et al. 1998, 1999). Neither vitronectin nor RGD peptides had any effect on hantavirus infection of these cells, although β_3-specific sera still blocked infectivity, indicating that the β_3 integrin mutant conferred cell susceptibility to viral infection (GAVRILOVSKAYA et al. 1998). These findings together with the ligand specificity of hantavirus–integrin interactions imply that non-RGD ligand contacts block hantavirus–integrin interactions or that specific integrin vitronectin or integrin fibronectin conformations are unable to interact with hantaviruses.

Interestingly, the $\alpha_v\beta_3$ integrin ligand vitronectin, but not other RGD containing ligands, blocks hantavirus infection. Regions outside of the RGD binding site have been shown to specify binding of fibronectin, fibrinogen, and von Willebrand factor (SUEHIRO et al. 1996; TOZER et al. 1996a,b; SUGIMORI et al. 1997; TOZER et al. 1999). Although this site does not mediate β_3-vitronectin interactions, a ligand-specific interactive region is likely to be present in β_3 integrins, which confers high affinity vitronectin binding by the receptor (TOZER et al. 1996; SUGIMORI et al. 1997). As a result, it is likely that hantaviruses engage a vitronectin binding domain of β_3 integrins or that the dimeric vitronectin sterically blocks hantavirus interactions with β_3 integrins (SMITH and CHERESH 1988; FRELINGER et al. 1990; MARKS et al. 1991; LOSKUTOFF et al. 1999; SCHVARTZ et al. 1999).

8.7 Hantavirus Growth

Hantaviruses grow slowly in tissue culture to maximal titers of approximately 5×10^6FFU/ml (focus forming units per milliliter). Because of the potential for human infection, BSL-3 (biosafety level-3) facilities are required for tissue culture growth of HPS and HFRS strains, and rodent infections require BSL-3 or BSL-4 facilities. Reverse genetic systems have not been established for hantaviruses, and this currently prevents the modification of hantaviruses through recombinant approaches. The lack of reverse genetics, difficulties in studying the heterodimeric G1 and G2 surface glycoproteins of the virus, and the divergence of hantavirus glycoproteins (40–60% divergent) has hampered studies aimed at defining the viral attachment protein.

8.8 Hantavirus Cultivation Does Not Select for Integrin-Specific Viral Variants

Although it is possible that adapting hantaviruses to growth in Vero E6 cells could have selected for hantaviruses that gain entry via β_3 integrins, this is very unlikely. Two reports demonstrate, by passaging hantaviruses in tissue culture, that hantavirus proteins are unaltered (PLYUSNIN et al. 1994; CHIZHIKOV et al. 1995). In one study, the hantavirus genome was sequenced in its entirety from a patient, a small mammal host, and following passage five times in Vero E6 cells, and no amino acid sequence differences were observed in any viral protein (CHIZHIKOV et al. 1995). Although changes were detected in non-coding regions, this demonstrates that viral variants were not selected to use $\alpha_v\beta_3$ integrins by viral adaptation or passage. It has now been demonstrated that five separate hantaviruses, HTN, SEO, PUU, SNV, and NY-1 with up to 60% divergent amino acid sequences in their surface glycoproteins, enter cells via β_3 integrins and are effectively blocked by only one integrin ligand, vitronectin. Similarly, the infectivity of three non-pathogenic hantaviruses is blocked by β_1-specific sera and the β_1 integrin ligand fibronectin

(Gavrilovskaya et al. 1998, 1999; Mackow et al. 1999). As a result, hantavirus use of β_3 integrins corresponds directly with the pathogenic potential of the virus. The question remaining is how does β_3 integrin usage contribute to hantavirus pathogenesis?

8.9 β_3 Integrins Regulate Capillary Integrity

The potential for pathogenesis to be effected by altering the host state of β_3 integrins is high, and even small differences in the binding affinity or specificity of these receptors may vary the response. β_3 integrins are prominent cell surface receptors on endothelial cells and platelets that mediate platelet activation, endothelial cell adherence, and regulate capillary integrity (Cheresh 1987; Hawiger 1995; Gerber et al. 1996; Halvorson et al. 1996; Hynes et al. 1996; Suehiro et al. 1996; Sugimori et al. 1997; Hynes et al. 1999; Hynes and Hodivala-Dilke 1999; Veinot et al. 1999). β_3 integrins include two members, $\alpha_{IIb}\beta_3$ (CD41/CD61) and $\alpha_v\beta_3$, (CD51/CD61) which mediate a diverse group of cellular interactions and signaling responses (Cheresh 1987; Hynes 1992; Shattil et al. 1994; Schwartz et al. 1995; Hynes et al. 1999; Hynes and Hodivala-Dilke 1999). $\alpha_{IIb}\beta_3$ receptors are found on platelets where they mediate and regulate platelet activation and participate in thrombus formation. $\alpha_v\beta_3$ is found on platelets, a number of immune cells, and endothelial cells, and mediates interactions with a variety of cells and ligands (Charo et al. 1990; Sugimori et al. 1997).

β_3 integrins on endothelial cells and platelets play central roles in maintaining capillary integrity (Cheresh 1987; Hawiger 1995; Gerber et al. 1996; Halvorson et al. 1996; Hynes and Wagner 1996; Suehiro et al. 1996; Sugimori et al. 1997; Wu et al. 1998; Hynes et al. 1999; Hynes and Hodivala-Dilke 1999; Veinot et al. 1999). Alterations in $\alpha_v\beta_3$ integrin binding permits increased transcapillary fluid fluxes and alter the hydrodynamics of capillaries (Hawiger 1995; Tsukada et al. 1995; Mogford et al. 1996; Hodivala-Dilke et al. 1999; Hynes et al. 1999; Hynes and Hodivala-Dilke 1999). Further, $\alpha_v\beta_3$ and $\alpha_5\beta_1$ differentially regulate arteriolar smooth muscle, resulting in vasodilation or vasoconstriction, respectively, through the intracellular activation of calcium channels (Charo et al. 1990; Hawiger 1995; Tsukada et al. 1995; Mogford et al. 1996; Sugimori et al. 1997; Hodivala-Dilke et al. 1999; Hynes et al. 1999; Hynes and Hodivala-Dilke 1999).

8.10 Role of Vitronectin Interactions in Pathogenesis

$\alpha_v\beta_3$ integrins were originally described as cellular vitronectin receptors (Charo et al. 1990; Sugimori et al. 1997). The use of the vitronectin receptor by hantaviruses and the ability of vitronectin to block hantavirus infectivity also suggests the potential for altered regulation of vitronectin to contribute to viral pathogenesis. Vitronectin is an adhesive glycoprotein present in the blood in an inactive complex

with plasminogen activator inhibitor I (PAI-1) (Loskutoff et al. 1999; Schvartz et al. 1999; Veinot et al. 1999; Wohn et al. 1999). Although vitronectin is present in the blood, the vitronectin RGD is cryptic (Seiffert and Smith 1997) and incapable of binding integrins unless fractionated, activated by PAI-1 in areas of tissue injury or thrombosis, or complexed with thrombin-antithrombin III or complement (C5b–C9) (Loskutoff et al. 1999; Schvartz et al. 1999; Wohn et al. 1999). These interactions result in disulfide-linked multimers of vitronectin and expose heparin binding and RGD binding sites on vitronectin for binding to β_3 integrins (Seiffert and Smith 1997).

The vitronectin-PAI-1 complex stabilizes both PAI-1 and vitronectin and complexed PAI-1 is a thrombin inhibitor (Loskutoff et al. 1999; Schvartz et al. 1999; Wohn et al. 1999). PAI-1 is itself a complex regulatory molecule present in at least five forms that are serine protease inhibitors and anti-adhesive factors in platelet attachment (Loskutoff et al. 1999). Vitronectin binds to PAI, $\alpha_v\beta_3$ integrins, and urokinase plasminogen activator receptor (uPAR) and as a result, it participates in the dynamic regulation of fibrinolysis and cellular adhesion (Loskutoff et al. 1999; Schvartz et al. 1999; Wohn et al. 1999). Vitronectin also inhibits complement mediated cell lysis and competes with heparin binding to antithrombin III, preventing rapid activation of thrombin and thereby functioning in both complement and coagulation systems (Loskutoff et al. 1999). Although it is not clear how these functions are coordinated or regulated, vitronectin provides a unique link between cell adhesion and serum proteolytic activation cascades that could participate in hantavirus-induced thrombocytopenia, increased vascular permeability, or hemorrhage.

8.11 Involvement in Platelet Function

Hantavirus infections of humans result in acute thrombocytopenia. The use of β_3 integrins by hantaviruses and the role of these receptors in platelet activation and recruitment, as well as potential roles for vitronectin and PAI-1 in regulating platelet function, provide a rationale for platelet dysfunction during pathogenic hantavirus infection. Interestingly, humanized monoclonal antibodies, which are used clinically to block platelet aggregation, also block pathogenic hantavirus infectivity. This suggests that hantavirus use of β_3 integrins may dysregulate normal platelet adhesion and activation responses and contribute to thrombocytopenia and vascular permeability changes observed in hantavirus disease.

8.12 Glanzmann's Disease

The involvement of β_3 integrins in vascular disease is prominently documented in a clinical bleeding disorder referred to as Glanzmann's thrombasthenia (Baker et al. 1997; Hynes et al. 1999; Hynes and Hodivala-Dilke 1999). Glanzmann's disease is a rare genetic disease of humans characterized by clotting abnormalities and

cutaneous hemorrhage (BAKER et al. 1997). These deficits result from integrin-specific mutations or deletions within the $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ integrins, which dysregulate platelet aggregation, clot retraction, and endothelial cell adherence properties. Prolonged clotting times, impaired platelet activation, and microvascular bleeding are the primary elements of Glanzmann's disease (HYNES and HODIVALA-DILKE 1999). The role of β_3 integrins in Glanzmann's disease suggests a direct means by which viral interaction with β3 integrin receptors may participate in hantavirus pathogenesis.

8.13 β_3-Integrin Knockout Mice Mimic Glanzmann's Disease

A mouse model of human Glanzmann's disease has been developed by generating β_3-integrin subunit knockout mice (murine $\beta_3^-/-$) (HODIVALA-DILKE et al. 1999). These mice show nearly identical defects to those of human Glanzmann's disease. Although mice are viable, they have prolonged clotting times and impaired platelet activation, resulting in cutaneous vascular hemorrhage, which leads to reduced survival. These mice demonstrate the direct effect of β_3 integrins on platelet function and vascular permeability, and they illustrate the dramatic role β_3 integrins can play in vascular disease (HODIVALA-DILKE et al. 1999). The fact that hantaviruses use β_3 integrins and similar-to-β_3-integrin knockouts, cause thrombocytopenia, and alter vascular permeability (hemorrhage or edema) during infection suggests that β_3-integrin usage is likely to play a central role in the hantavirus disease process.

8.14 β_3 Integrins Contain Species-Specific Differences

The interaction of hantaviruses with integrins from their animal hosts has not been studied, and there is little or no information available about β_3 integrins from these animals. Sequences of β_3 integrin subunits from humans, mice (*Mus musculus*), dogs, pigs, rats, rabbits, and chickens are present in GenBank. Human β_3-integrin sequences differ from these species by ≤15%: rabbit and dog (4%), pig (7%), rat (9%), mouse (10%), and chicken (15%). Human and murine β_3 integrins diverge by 10% at the protein level and include unique residue changes within the metal ion dependent activation site (MIDAS), which could contribute to differential hantavirus interactions (TOZER et al. 1996). Rats are hosts for SEO hantaviruses, yet rat β3 integrin sequences differ from human β3s by nearly as much as the mouse integrin, albeit primarily at unique residues from that of the mouse. An argument can be made that host β3 integrins are not likely to be host receptors for hantaviruses, since no disease results from hantavirus infection of their hosts. However, the evolution of hantaviruses with their hosts may also have selected for non-pathogenic hantavirus-integrin interactions in animal hosts. In fact, the relatively high conservation of β3-integrin subunits, the divergence of hantavirus glycoproteins, and the ability of

human β3 integrins to confer cell susceptibility to pathogenic hantaviruses suggests that host β3 integrins are likely to serve as cellular receptors for pathogenic hantaviruses.

8.15 Human, But Not Murine, β_3 Integrins Confer Cell Susceptibility

The inability of hantaviruses to infect adult laboratory mice (*Mus musculus*) and the availability of a recombinant murine β_3 integrin, permitted a test of whether hantavirus host range restriction in mice was at the level of the integrin receptor. CHO cells transfected with plasmids expressing murine or human β3 integrins, in the presence or absence of co-transfected human α_v subunits, were assayed for their susceptibility to hantavirus infection (Fig. 6). Murine β_3 integrin subunits form heterodimers with human α_v subunits that are trafficked to the cell surface. Similarly, human β_3 integrins are transported to the cell surface with the hamster α_v subunit present in CHO cells (SUGIMORI et al. 1997). The introduction of human β_3 integrins into CHO cells permitted infection by NY-1 or HTN viruses, as previously demonstrated (GAVRILOVSKAYA et al. 1998; GAVRILOVSKAYA et al. 1999; MACKOW et al. 1999). Interestingly, cells transfected with the murine β_3 integrin failed to confer cell susceptibility to hantavirus infection (Fig. 6). It has also been shown that transfecting human β_3 integrins into murine cells confers cell susceptibility to hantavirus infection and that human α_v subunits have no effect. These findings suggest that differences between human and murine β_3 integrins determine cellular susceptibility to hantavirus infection and permit the use of recombinant approaches to define domains and residues which are required for infection by pathogenic hantaviruses.

Experiments with recombinant integrins indicate that murine β_3 integrins are insufficient to confer cell susceptibility to pathogenic hantaviruses and that human

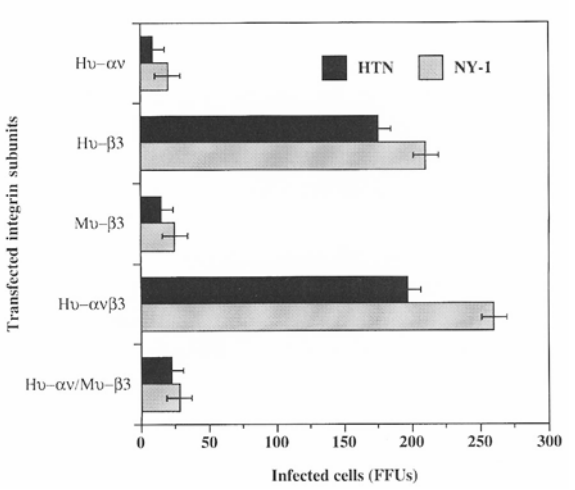

Fig. 6. Human but not Murine β_3 integrins render CHO cells permissive to pathogenic hantaviruses. CHO cells were transfected with human or murine β_3 with and without the human α_v integrin subunits. Transfected cells were infected with NY-1 or HTN 36h post-transfection, and infected cells were quantitated as in Fig. 1 24h post-infection

β_3 integrins supplant murine β_3 deficiencies. These findings suggest: (a) that species specific β_3 integrins are likely to be determinants of hantavirus host range; (b) that human β_3 integrin transgenic mice should confer susceptibility to infection by pathogenic hantaviruses; and (c) that human β_3 integrin transgenic mice have the potential to serve as animal models of hantavirus disease. An analogous situation has been reported for poliovirus, where the human, but not murine, poliovirus receptor confers cell susceptibility, tissue tropism, host range restriction and pathogenesis (MENDELSOHN et al. 1986; MENDELSOHN et al. 1989; RACANIELLO 1991, 1996; MORRISON et al. 1992; REN and RACANIELLO 1992; RACANIELLO et al. 1993; BIBB et al. 1994; RACANIELLO and REN 1994; COLSTON et al. 1995; TAFFS et al. 1996; GROMEIER et al. 1997; SUGIMORI et al. 1997).

8.16 Hantavirus Pathogenesis

The mechanism of hantavirus pathogenesis is still unknown although accumulating data suggest that β_3 integrins are likely to be key elements of hantavirus disease. The means by which some hantaviruses effect hemorrhage or pulmonary edema may stem from virus-specific differences in receptor interactions, alterations in intracellular signaling, the specific induction of cytokines, or the differential regulation of additional platelet or endothelial cell receptors. Large differences in the glycoprotein composition of HFRS- and HPS-causing hantaviruses (approximately 60% and 40% unique residues in G1 and G2 proteins, respectively), further suggest how differential pathogenic responses could be effected by individual viruses despite common β_3 integrin usage. Tumor necrosis factor (TNF) levels in patient sera have been suggested to play a role in hantavirus pathogenesis (MORI et al. 1999) and complement activation has also been suggested to contribute to HFRS disease.

8.17 Lack of Animal Model for Hantavirus Disease

The highly lethal nature of hantavirus infections of humans and the lack of an animal model of hantavirus disease has hampered our ability to study hantavirus pathogenesis. The ability of human β_3 integrins to confer cell susceptibility to hantavirus infection suggests that animal models for hantavirus infection and pathogenesis are plausible. The presence of murine β_3 integrins is likely to ameliorate any disease that might be induced within human β_3 integrin transgenic animals. However, a cross of human β_3 integrin transgenics with murine β_3-integrin knockout mice should both reconstitute β_3 integrin function within the knockouts, by providing the human β_3 subunit, and permit hantavirus infection of murine endothelial cells (HODIVALA-DILKE et al. 1999). If as suggested, the β_3 integrins are primary elements of hantavirus pathogenesis, the human $\beta_3^{+/+}$; murine $\beta_3^{-/-}$ mice are likely be models of hantavirus pathogenesis and permit studies of hantavirus-directed immune responses and therapeutic interventions for hantavirus disease.

8.18 Hantaviruses Regulate β_3 Integrin Function

One of the primary functions of endothelial cells in maintaining capillary integrity and vascular hemostasis is their ability to migrate on extracellular matrix proteins. The ability of β_3 integrins to confer cell susceptibility to hantaviruses and for vitronectin to inhibit hantavirus cell interactions suggested that integrin mediated endothelial cell functions may be regulated by pathogenic hantaviruses. Using FACS analysis the number of β_1 and β_3 integrin receptors on the surface of endothelial cells increased or did not change during infection by NY-1 or PH viruses. However, when the effect of pathogenic (NY-1) and non-pathogenic (PH) hantaviruses on endothelial cell migration was tested, a dramatic difference in cellular response was detected. NY-1 infected cells were able to migrate normally on collagen but migration was inhibited (> 60%) on vitronectin (Fig. 7). In contrast, the migration of PH infected cells was increased on collagen and identical to uninfected cells on a vitronectin matrix (Fig. 7). These initial findings suggest that β_3 integrin mediated endothelial cell functions are altered by pathogenic, but not non-pathogenic, hantaviruses and suggest at least one means by which pathogenic hantaviruses may alter vascular permeability during infection.

9 Summary

Hantaviruses cause two potentially lethal diseases, HPS and HFRS, and both diseases result in defects in vascular permeability and platelet function. Human β_3 integrins confer cellular susceptibility to HPS- and HFRS-causing hantaviruses, a fact directly linking platelets, endothelial cells, and hantavirus diseases to the use of

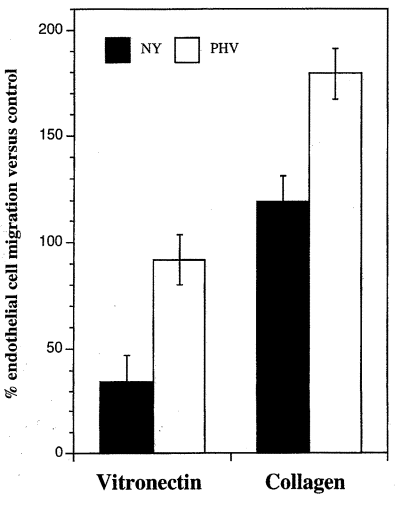

Fig. 7. Hantavirus regulation of endothelial cell migration. Human umbilical vein endothelial cells (HUVECs) were infected at an MOI of 1 with NY-1 or PH viruses. Infected cells were detached from monolayers using enzyme free cell dissociation buffer (GIBCO) and plated on vitronectin or collagen-coated polycarbonate Transwell membranes with 8-μ pores in EBM-2 media (Clonetics). HUVEC migration through pores was quantitated after 3h at 37°C, fixed with DifQuick stain (Dade Bering), and quantitated. Cell migration is presented as a percentage of control uninfected migrated cells. Experiments are representative of three independent experiments

cellular receptors that maintain capillary integrity and regulate platelet function. The role of vitronectin, PAI-1, uPAR, and complement cascades in hantavirus pathogenesis are unstudied but may contribute to specific disease syndromes effected by hantaviruses.

The divergence of hantavirus surface glycoproteins and common β_3-integrin usage provides further insight into the interaction of hantaviruses with cells. G1 and G2 glycoprotein variation is likely to contribute to additional interactions that determine pathogenic responses to individual viruses. β_3-integrin usage also suggests that common elements exist on G1 or the more highly conserved G2 surface glycoprotein, which mediate viral attachment to integrins. Although there is currently no data defining the virion attachment protein, the development of antibodies that recognize the hantavirus attachment protein and block integrin interactions is of interest since it is likely to provide an additional point for therapeutic intervention and vaccine development.

There are a plethora of effects that could be elicited by hantavirus regulation of cellular β_3 integrins and their ligands that are consistent with hantavirus diseases. Since β_3 integrins are critical adhesive receptors on platelets and endothelial cells and regulate both vascular permeability and platelet activation and adhesion, the use of these receptors by hantaviruses is likely to be fundamental to hantavirus pathogenesis. The lack of an animal model for hantavirus pathogenesis has prevented a systematic analysis of immune and cellular responses to hantavirus infections, and it impedes our ability to study protective or therapeutic approaches to hantavirus diseases. However, recent findings suggest that human β_3 integrins within transgenic mice may provide animal models of hantavirus pathogenesis and have the potential to radically alter the ability to investigate hantavirus disease.

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