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β_3 integrins mediate the cellular entry of hantaviruses that cause respiratory failure

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ABSTRACT **Newly emerged hantaviruses replicate primarily in the pulmonary endothelium, cause acute platelet loss, and result in hantavirus pulmonary syndrome (HPS). We now report that specific integrins expressed on platelets and endothelial cells permit the cellular entry of HPS-associated hantaviruses. Infection with HPS-associated hantaviruses, NY-1 and Sin Nombre virus (SNV), is inhibited by antibodies to β_3 integrins and by the β_3-integrin ligand, vitronectin. In contrast, infection with the nonpathogenic (no associated human disease) Prospect Hill virus was inhibited by fibronectin and β_1-specific antibodies but not by β_3-specific antibodies or vitronectin. Transfection with recombinant $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ integrins rendered cells permissive to NY-1 and SNV but not Prospect Hill virus infection, indicating that $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ integrins mediate the entry of NY-1 and SNV hantaviruses. Furthermore, entry is divalent cation independent, not blocked by arginine-glycine-aspartic acid peptides and still mediated by, ligand-binding defective, $\alpha_{IIb}\beta_3$-integrin mutants. Hence, NY-1 and SNV entry is independent of β_3 integrin binding to physiologic ligands. These findings implicate integrins as cellular receptors for hantaviruses and indicate that hantavirus pathogenicity correlates with integrin usage.**

Hantaviruses define a unique genus of segmented negative-stranded RNA viruses. Hantaviruses are structurally formed by two highly ordered integral membrane surface glycoproteins and internal nucleocapsid and RNA polymerase proteins (1). Each hantavirus persistently infects a primary small mammal host, and hantaviruses are spread to man through the inhalation of aerosolized excreted virus. In addition to hantaviruses for which no human disease has been observed, hantaviruses are known to cause two diseases, hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome (HPS).

In 1993, hantaviruses emerged as the cause of an acute and highly lethal (50%) respiratory distress syndrome in the Southwestern U.S. (2–6). Sin Nombre virus (SNV) was identified as the causative agent of Southwestern cases, and HPS has since been identified in 28 U.S. states, in Canada, and most recently in South America (7–9). Although endothelial cells are infected in a variety of organs, hantaviruses replicate predominantly in pulmonary endothelial cells and macrophages (3, 5, 10, 11). However, the means by which specific hantaviruses cause pulmonary or renal diseases is obscure (1, 3, 5). In humans, hantaviruses cause thrombocytopenia or platelet loss, and in HPS cases, acute pulmonary edema is observed (3, 5). However, there is little immune cell recruitment or damage to hantavirus-infected pulmonary endothelial cells (3, 5, 10).

Determinants of pathogenesis have not been defined for any hantavirus, although hantavirus interactions with endothelial cells and platelets are likely to mediate pathogenesis in man. Endothelial cells and platelets maintain capillary integrity and direct vascular repair and immune cell responses through specific cellular receptors. Integrins are heterodimeric receptors composed of a combination of α and β subunits, which specify cell–cell adhesion, immune cell recruitment, extravasation, platelet aggregation, and the migration of endothelial cells on extracellular matrix proteins (12–19). $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins are abundant surface receptors of endothelial cells and platelets (15–18), respectively, and are central to regulating platelet activation and maintaining capillary integrity (14, 20).

Specific cell surface proteins are capable of mediating viral attachment to cells or facilitate viral entry into cells. However, receptors that mediate the cellular attachment and entry of hantaviruses or other Bunyaviridae have not been defined. In this report, we investigate the interactions of HPS-associated hantaviruses with Vero E6 and endothelial cells as well as CHO cells transfected with recombinant integrins. Our results indicate that β_3 integrins facilitate the cellular entry of pathogenic HPS-associated hantaviruses.

MATERIALS AND METHODS

Cell and Virus. Vero E6 and CHO cells were grown in DMEM, 10% fetal calf serum, L-glutamine, and penicillin-streptomycin (GIBCO). CHO cells were supplemented with 100 nM nonessential amino acids (GIBCO). Human umbilical vein endothelial cells (HUVECs) were grown in endothelial cell basal medium-2 (Clonetics, San Diego) with 0.1% endothelial cell growth factor. CHO cells transfected with integrins $\alpha_{IIb}\beta_3$, (CHO-A5), $\alpha_v\beta_3$ (CHO-VNRC), and a ligand-defective $\alpha_{IIb}\beta_3$ mutant (CHO-BCC4) were described (21, 22). Biosafety level 3 (BSL-3) facilities were used throughout these experiments for the growth of three hantaviruses: SNV (CC107 isolate, passage 6), NY-1 (passage 7), and Prospect Hill virus (PHV). SNV and NY-1 are distinct hantaviruses associated with HPS (23, 24). PHV has not been associated with any human disease (10, 25).

Ligands, Peptides, and Antibodies. Vitronectin, fibronectin, laminin, fibrinogen, heparin, phytohemagglutinin, dextran sulfate, chondroitin sulfate, BSA, and glycophorin A were purchased from Sigma. G*RGD*SP or G*RGE*SP peptides were

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Abbreviations: HPS, hantavirus pulmonary syndrome; SNV, Sin Nombre virus; CHO, Chinese hamster ovary; FFUs, focus-forming units; N-protein, nucleocapsid protein; RGD, arginine-glycine-aspartic acid; HUVEC, human umbilical vein endothelial cell.

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purchased from GIBCO. Polyclonal rabbit sera to β_1, β_3, β_4, α_1, α_2, α_5, and α_V and polyclonal goat sera to $\alpha_5\beta_1$ (blocking) as well as blocking mAbs to β_2 (mAb 1962) and $\alpha_v\beta_3$ (mAb 1976) were purchased from Chemicon. Antibodies were used at a range of concentrations (20 ng/ml–40 μg/ml) in addition to those within figures. After washing, a 1:2000 dilution of anti-mouse or anti-rabbit sera was incubated with cells for 1 hr at 37°C.

The NY-1 S segment (26) was cloned into the pET30a (Novagen) plasmid at the *Bam*HI site, and the nucleocapsid protein (N-protein) was expressed in *Escherichia coli* after 1 mM isopropyl β-D-thiogalactoside induction of plasmid-transformed BL-21(DE3) bacteria. N-protein was purified by nickel affinity chromatography by using Ni-nitrilotriacetic acid resin as described by manufacturer's instructions (Qiagen). Polyclonal rabbit anti-nucleocapsid protein was made by hyperimmunizing rabbits with bacterially expressed and nickel affinity-purified NY-1 N-protein.

Quantitation of Hantavirus-Infected Cells. Cells were pretreated with antibodies or potentially competitive ligands (1 hr, 37°C) before hantavirus addition. Sera were removed, and ≈200–800 hantavirus focus-forming units (FFUs) were adsorbed to Vero E6 cells in duplicate wells of a 96-well plate (1 hr, 37°C). Viral inocula were removed, monolayers washed, and cells were further incubated 24–36 hr before methanol fixation. Methods for immunoperoxidase staining of viral antigens in infected cells have been described (27). In brief, cell monolayers were methanol fixed (100%, 10 min, 4°C), incubated with polyclonal rabbit anti-nucleocapsid sera (1/2,000, 1 hr, 37°C), and subsequently incubated with goat anti-rabbit horseradish peroxidase conjugates (1/5,000). Infected cells were quantitated after staining with 3-amino-9-ethylcarbazole (0.026%) in 0.1 M sodium acetate (pH 5.2) and 0.03% H_2O_2 (27).

RESULTS

Because hantaviruses replicate in endothelial cells and impact platelet and endothelial cell function, we hypothesized that platelet or endothelial cell receptors may be involved in the cellular entry of hantaviruses. We assayed the ability of ligands for platelet or endothelial cell receptors to inhibit NY-1, SNV, or nonpathogenic PHV infections of Vero E6 or HUVECs. We tested the ability of fibrinogen, heparin, and extracellular matrix proteins vitronectin, fibronectin, and laminin to inhibit NY-1 infectivity (Fig. 1*A*). Pretreatment of cells with vitronectin significantly reduced NY-1 or SNV (not shown) infection of HUVEC or Vero E6 cells. In contrast, the PHV infection of Vero E6 cells was inhibited (>80%) by fibronectin but not by vitronectin (Fig. 1*B*). This result suggested that pathogenic HPS-associated hantaviruses and nonpathogenic hantaviruses might enter cells using different cell surface components. Furthermore, this suggested that an integrin–vitronectin receptor might mediate the entry of pathogenic NY-1 and SNV hantaviruses.

To determine whether integrins are involved in hantavirus infection, we pretreated cells with mAbs or polyclonal antibodies and subsequently adsorbed ≈400 FFUs of NY-1, SNV, or PHV hantaviruses to monolayers. Antibodies to α_1, α_2, α_5, $\beta1$, $\beta2$, $\beta4$, and $\alpha_5\beta1$ integrins (Fig. 2) and platelet-endothelial cell adhesion molecule (PECAM)-1, E-cadherin, and β-catenin (not shown) had no apparent effect on NY-1 infectivity even when cells were pretreated with 40 μg/ml mAbs or polyclonal antibodies.

Pretreatment of Vero E6 (Fig. 2) (2–40 μg/ml) or HUVECs (Fig. 3) (20 ng/ml–20 μg/ml) with mAbs or polyclonal antibodies to $\beta3$ integrins specifically inhibited NY-1 or SNV infectivity by 60–70% and is titratable (Fig. 3). In addition, mAb to $\alpha_v\beta_3$ reduced NY-1 infectivity by 70%. Antibodies to α_v also slightly reduced NY-1 infectivity (>40%) but not SNV or PHV infectivity. In contrast, PHV infections were unaffected by antibodies to β_3 or $\alpha_v\beta_3$ but were inhibited by β_1 and

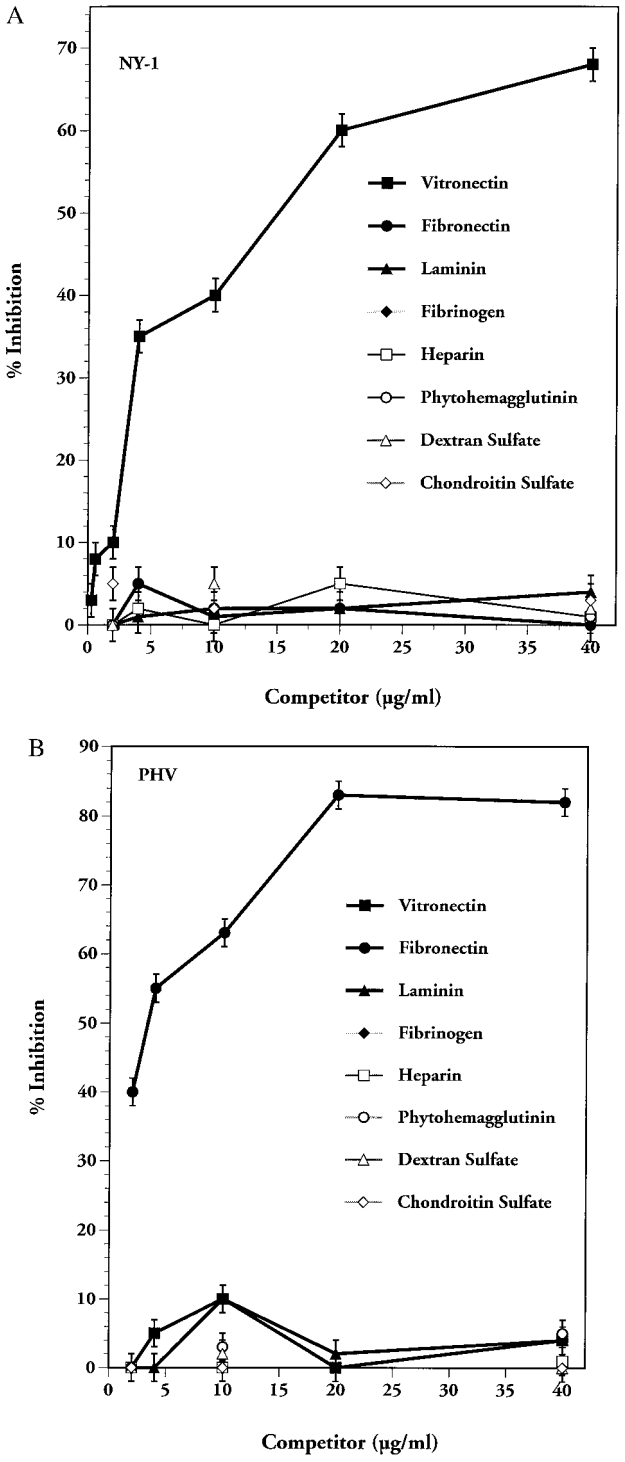

FIG. 1. (*A* and *B*) Ligand-specific inhibition of hantavirus infectivity. Vero E6 cells were pretreated with potentially competitive ligands for 1 hr before viral adsorption. Approximately 400 FFUs of NY-1 (*A*) or PHV (*B*) hantaviruses were adsorbed to duplicate wells of a 96-well plate. After adsorption, inocula were removed and cells were washed and further incubated 24–36 hr before methanol fixation. Hantavirus-infected cells were immunoperoxidase stained as described (27) by using polyclonal rabbit anti-nucleocapsid sera made to bacterially expressed and Nickel affinity purified NY-1 N-protein. Infected cells were quantitated and compared with control infections without competitor proteins. Results are presented as the percentage of inhibition of control infections. BSA and glycophorin A did not affect NY-1 or PHV infectivity (not shown).

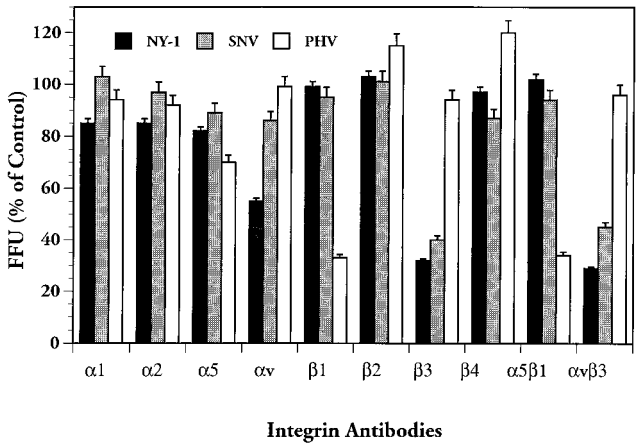

FIG. 2. Hantavirus infectivity is inhibited by integrin-specific antibodies. Duplicate wells of Vero E6 cells were pretreated for 1 hr (37°C) with 20 μg/ml of antibodies to specific integrins and after PBS washing, were similarly incubated with a 1:2000 dilution of anti-rabbit or anti-mouse sera. Monolayers were washed and NY-1, SNV or PHV hantaviruses were subsequently adsorbed. Infected cells were quantitated as in Fig. 1. FFUs observed 36 hr. postinfection are expressed as a percentage of control infections for each viral inoculum. Polyclonal rabbit sera to β_1, β_3, β_4, α_1, α_2, α_5, α_V, and $\alpha_5\beta_1$ (blocking) as well as blocking mAbs to β_2 (mAb 1962) and $\alpha_v\beta_3$ (mAb 1976) were from Chemicon.

$\alpha_5\beta_1$-specific antibodies (70%) (Fig. 2). $\alpha_v\beta_3$ and $\alpha_5\beta_1$ have high affinities for vitronectin and fibronectin, respectively, although both receptors bind other proteins (28). These findings are consistent with the pattern of inhibition by integrin ligands (Fig. 1 *A* and *B*) and further suggest that pathogenic and nonpathogenic hantaviruses use different integrins for cellular entry.

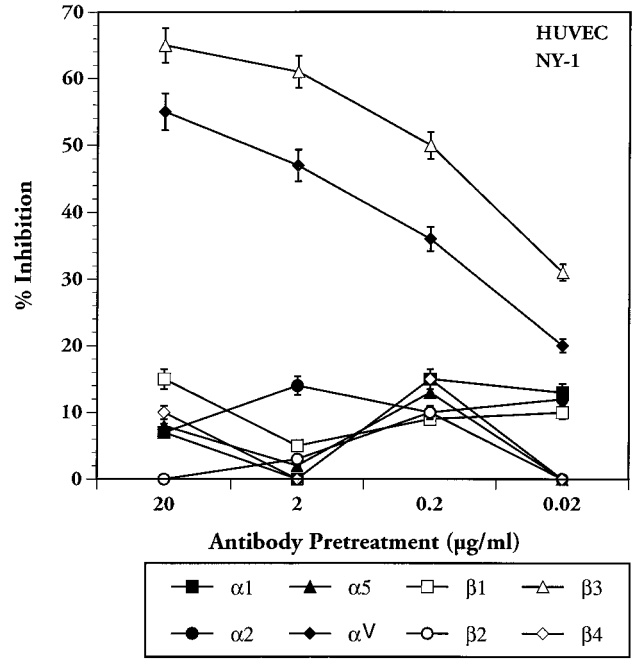

FIG. 3. Infection of HUVECs by NY-1 is inhibited by integrin-specific antibodies. HUVECs were pretreated with 20 ng/ml–20 μg/ml of integrin-specific antibodies for 1 hr as described in Fig. 2. After primary antibody addition (1 hr, 37°C) and PBS washing, a 1/2,000 dilution of anti-rabbit or anti-mouse sera, respectively, was incubated with cells (1 hr, 37°C). Infected cells were quantitated as in Fig. 1, and results are presented as the percentage of inhibition of control infections.

Recombinant $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ Integrins Promote NY-1 and SNV Infection of CHO Cells. CHO cells expressing recombinant integrin heterodimers were used to determine whether β_3 integrins were required for the cellular entry of hantaviruses (21). We inoculated CHO cells or CHO cells expressing recombinant $\alpha_v\beta_3$ (CHO-VNRC) or $\alpha_{IIb}\beta_3$ (CHO-A5) integrins and quantitated hantavirus infections. CHO cells expressing human $\alpha_{IIb}\beta_3$ integrins were permissive for SNV and NY-1 (not shown) but not PHV, resulting in the infection of virtually 100% of the monolayer and titers of 1×10^5 FFUs 7 days postinfection (SNV) (Fig. 4*A*). Quantitation of infected foci (Fig. 4*B*) showed that the number of NY-1- and SNV-infected cells increased 19- to 39-fold in CHO cells expressing $\alpha_{IIb}\beta_3$ and that $\alpha_v\beta_3$ also promoted infection (83% of Vero E6 FFU). There was a 4-fold increase in SNV- and a 30-fold increase in NY-1-infected CHO-VNRC cells (Fig. 4*B*) compared with CHO cells alone. There was no increase in the number of PHV-infected cells in either of the integrin-transfected cell lines and viral titers were not detected following infection (<10 FFU/ml).

A small number of CHO cells are reproducibly infected by NY-1, SNV, and PHV hantaviruses (0.3%–5% of identical FFUs in Vero E6 cells). This background is not reduced by the ligands or antibodies used in Figs. 1*A* or 2. Background infectivity appears to be a function of the viral inoculum (particulate or aggregated virus) because the infection does not spread to new sites in CHO cell monolayers without recombinant integrins (Fig. 4*A*). In fact, the inability of CHO cells to propagate hantaviruses without β_3 integrins further demonstrates β_3 integrin use in cellular entry.

To confirm that entry of NY-1 and SNV is specifically dependent on the presence of human $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ integrins on the surface of transfected CHO cells, ligands (not shown) or antibodies were used to block NY-1 and SNV infection of these cells. Both vitronectin and β_3-specific antibodies inhibited SNV or NY-1 infection of CHO-A5 or CHO-VNRC (not shown) (Fig. 5*A*). Neither treatment reduced the background level of infected CHO cells (≈50 foci for SNV and 10 for NY-1). These findings demonstrate that $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ integrins mediate the cellular entry of NY-1 and SNV hantaviruses.

Vitronectin and fibronectin contain arginine-glycine-aspartic acid (RGD) sequences, which meditate interactions with $\alpha_v\beta_3$ and $\alpha_5\beta_1$, respectively (19, 28–31). However, the RGD sequence is absent from the G_1 or G_2 surface glycoproteins of all hantaviruses. Pretreatment of cells with G*RGD*SP or G*RGE*SP peptides (200 μg/ml–4 mg/ml) had no effect on the infectivity of any hantavirus strain whereas vitronectin (5 μg/ml) pretreatment inhibited infection of NY-1 or SNV by >60%. Addition of G*RGD*SP but not G*RGE*SP peptides (200 μg/ml) before vitronectin (5 μg/ml) abrogated the inhibitory effects of vitronectin on hantavirus infection (Fig. 5*B*). Furthermore, EDTA pretreatment of cells (0.5–8 mM), which blocks endogenous ligand binding to integrins, did not block hantavirus infectivity (not shown). Finally, CHO cells transfected with a ligand-binding defective integrin (BCC4: β_3 D 119->A) (22) were still able to mediate NY-1 and SNV hantavirus entry (similar to CHO-A5, Fig. 4*A*). Entry of CHO-BCC4 cells was inhibited by a β_3-specific antibody (mAb 15, 50 μg/ml) but not inhibited by prior addition of vitronectin (Fig. 5*B*). Thus, the physiological ligand-binding function of β_3 integrins is not required for hantavirus infectivity.

DISCUSSION

Integrins are heterodimeric receptors composed of a combination of α and β subunits, which specify interactions with extracellular ligands and mediate endothelial cell migration as well as platelet and cell–cell adherence (18, 19). The active

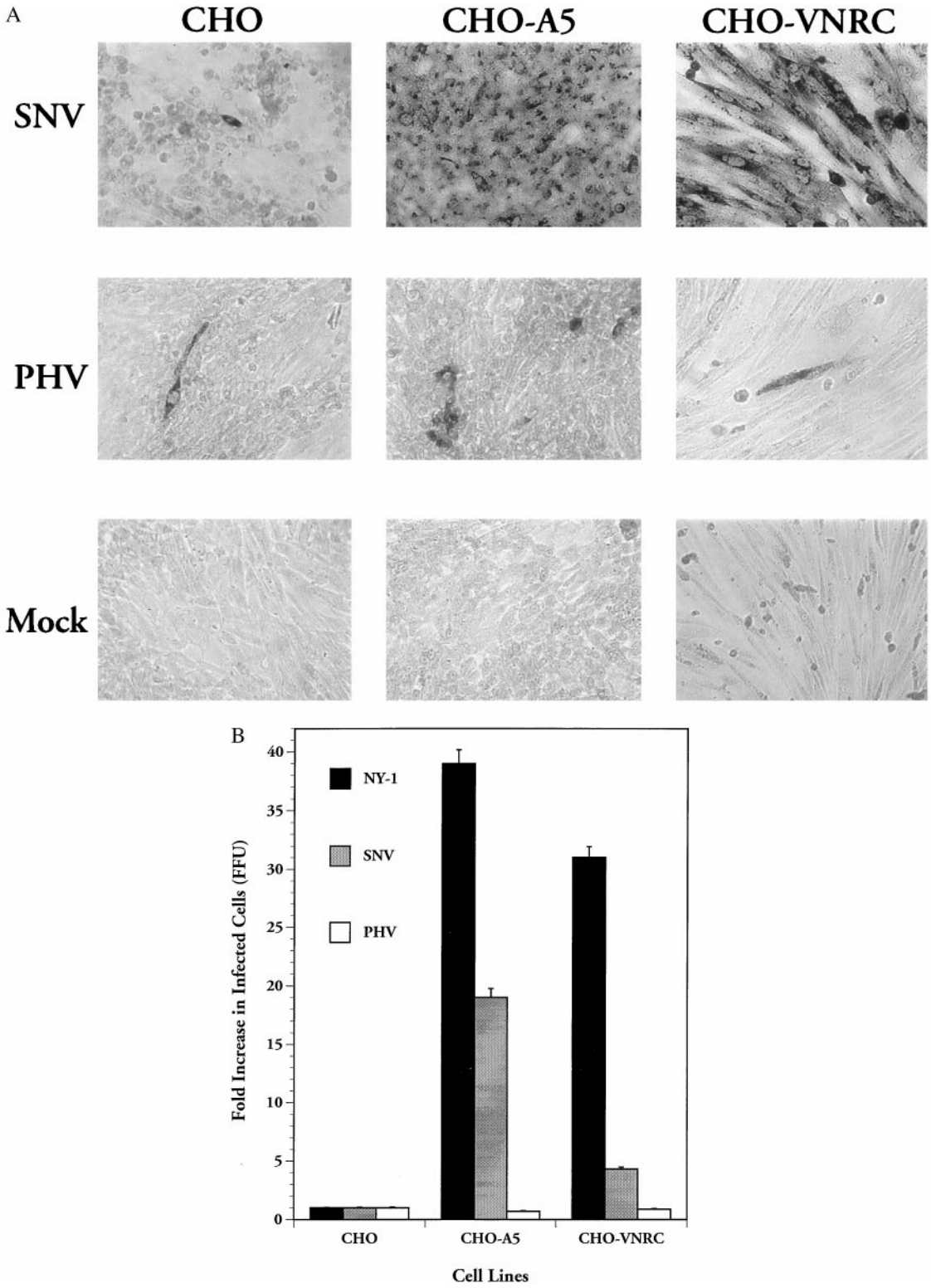

FIG. 4. (*A* and *B*) Recombinant integrins render CHO cells permissive to SNV and NY-1 hantaviruses. The ability of SNV (strain CC107) and PHV hantaviruses to infect CHO cells or CHO cells transfected with the $\alpha_{IIb}\beta_3$ integrin (CHO-A5 cells) or the $\alpha_v\beta_3$ integrin (CHO-VNRC) are depicted at 7 days postinfection (*A*). Representative pictures were taken from identically stained uninfected or hantavirus-infected cells (200 X magnification). The nucleocapsid protein present in infected cells was immunoperoxidase stained (dark brown precipitate) as described (27). (*B*) Quantitation of NY-1, SNV, and PHV hantavirus infection of CHO and CHO-integrin cell lines was determined 36 hr postinfection. The fold increase in the number of infected cells compared with immunoperoxidase-stained CHO cells is presented. Results were reproduced in six separate experiments.

state or availability of integrins on the surface of endothelial cells can alter the activation of integrin-specific intracellular signaling pathways, which regulate endothelial cell adherence (18, 32–35). Integrins are prominent endothelial cell and platelet receptors, and hantaviruses are known to infect pulmonary and other endothelial cells and cause thrombocytopenia in patients (3, 5, 10, 11). In this study, we demonstrate that integrins, which are central to maintaining microvascular

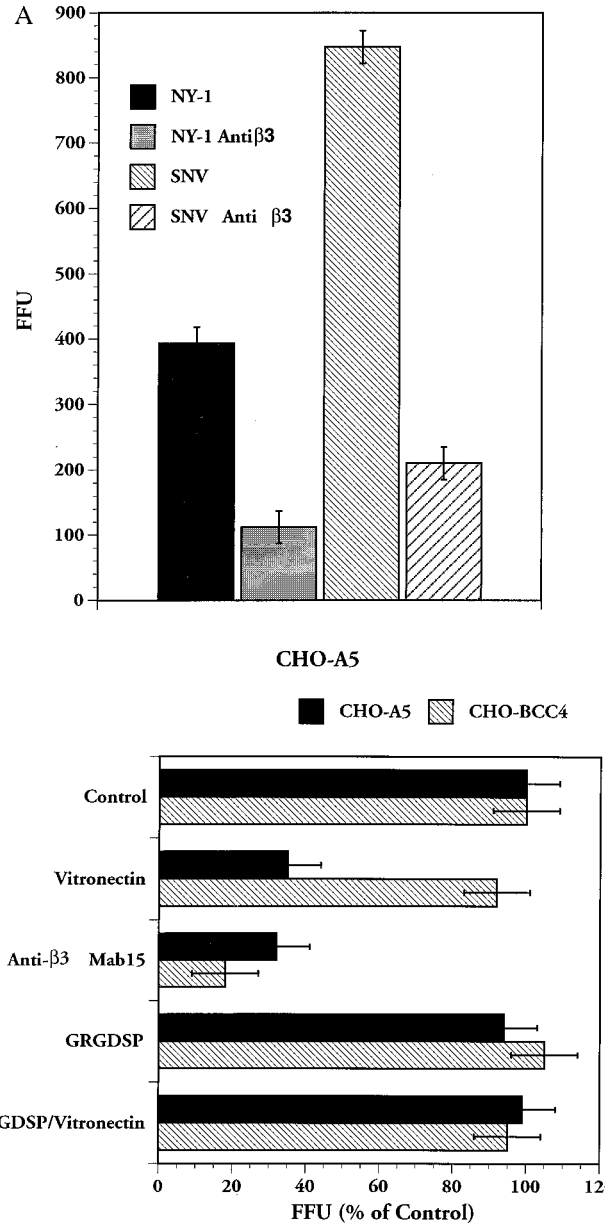

FIG. 5. (*A* and *B*) Specificity of Hantavirus Infection of CHO-A5 and CHO-VNRC Cells. (*A*) The number of FFUs quantitated after NY-1 or SNV inoculation of CHO-A5 cells is presented. Inoculation with or without prior treatment of cells with 20 μg/ml of rabbit anti-β3 polyclonal sera was performed as described in Fig. 2. Findings obtained with CHO-VNRC were nearly identical to those presented for CHO-A5. (*B*) CHO-A5 or CHO-BCC4 cells were inoculated in duplicate with NY-1 after pretreatment with potentially competitive ligands (1 hr, 37°C). As described in Fig. 1, infected cells were quantitated 36 hr postinfection and results are presented as a percentage of NY-1-infected CHO-A5 or CHO-BCC4 cells in the absence of ligand pretreatment. G*RGD*SP or G*RGE*SP (not shown) peptides (200 μg/ml–4 mg/ml), vitronectin (20 μg/ml), or β3-specific mAb 15 (50 μg/ml) was added as a potentially competitive inhibitor to 50-μl aliquots of NY-1 virus in duplicate wells during adsorption to 96-well plates. GRGDSP was added to monolayers 15 min before addition of vitronectin and subsequent virus adsorption in GRGDSP/vitronectin experiments.

barrier properties, facilitate hantavirus entry into cells (14, 20). These findings suggest that hantavirus–integrin interactions could participate in altering normal endothelial cell barrier functions.

Icosahedral viruses, which enter cells via integrins, are adenoviruses ($\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_M\beta_2$) (36, 37), papilloma viruses (α_6-containing integrins) (38), rotaviruses ($\alpha_2\beta_1$, $\alpha_4\beta_1$, and β_2 integrins) (39), foot-and-mouth disease virus (FMDV, $\alpha_v\beta_3$) (40), coxsackievirus A9 ($\alpha_v\beta_3$) (41), and echoviruses ($\alpha_2\beta_1$)(42, 43). Echovirus 1 is reported to bind to a unique domain of the $\alpha_2\beta_1$ integrin (43), whereas adenoviruses attach to an unknown cellular receptor and only a secondary interaction of the viral penton base with integrins facilitates cellular entry (37, 44).

Viruses that use $\alpha_v\beta_3$ integrins for entry interact with ligand-binding sites and are dependent on integrin recognition of a virally encoded RGD motif (36–38, 40, 41). Our findings demonstrate that NY-1 and SNV hantaviruses associate with $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins through unique RGD-independent interactions. NY-1 and SNV hantavirus infections are facilitated by the presence of $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins on cells and are inhibited by integrin-specific antibodies as well as the integrin ligand vitronectin. However, RGD motifs are absent from hantavirus proteins, RGD peptides fail to inhibit NY-1 and SNV infections and integrins, which are incapable of binding physiological ligands, facilitate NY-1 and SNV infection of CHO cells. This finding demonstrates the RGD-independence of the interaction and suggests that inhibitory effects of anti-integrin antibodies as well as vitronectin (SNV and NY-1) or fibronectin (PHV) are likely to alter integrin conformations required for hantavirus interaction or to sterically inhibit hantavirus interactions. This further suggests that hantaviruses interact with unique integrin regions or require more complex cell surface protein associations for cellular entry.

Antibodies to integrins did not completely block hantavirus entry in these studies even when added in large molar excess. However, the percentage of inhibition is within the range (63–74%) of integrin antibody inhibition of adenovirus, papilloma virus, FMDV, and coxsackie viruses (36–38, 40, 41). Papilloma virus studies also report that 10% of the observed binding was nonsaturable and likely to be nonspecific (38). This result could be similar to the residual hantavirus infectivity observed during infection of CHO cells. NY-1 and PHV also are inhibited 20–30% by the prebinding of some α subunit antisera (Fig. 2). As a result, it is possible that additional hantavirus interactions with α-integrin subunits, alternative cellular components, or coreceptors are required for hantavirus entry. Nevertheless, these findings indicate that specific integrins facilitate the entry of hantaviruses into endothelial cells and that different integrins confer the entry of pathogenic and nonpathogenic hantavirus strains.

These studies have not defined the requirements for PHV entry into cells. Antibodies to β_1 integrins and an integrin ligand, fibronectin, inhibit PHV infection of Vero E6 and endothelial cells. However, CHO cells contain $\alpha_3\beta_1$ and $\alpha_5\beta_1$ integrins but did not facilitate PHV infection. As with other hantaviruses, a residual infection of CHO cells was observed for PHV although infectivity was not enhanced by the presence of recombinant $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ integrins. Because both pathogenic and nonpathogenic hantaviruses replicate similarly in endothelial cells, this suggests that PHV infectivity may be dependent on the presence of additional α-integrin subunits or on additional cell surface proteins, which are not present in CHO cells.

Hantaviruses are generally adapted to growth on Vero E6 from their small mammal hosts, and cell culture adaptation could contribute to the hantavirus–integrin specificity reported here. It is also unclear whether passage of PHV in Vero E6 cells has contributed to integrin-specific differences between PHV and HPS-associated strains. However, both of these possibilities are unlikely. When entire SNV genomes were compared from (*i*) a patient (*ii*) *Peromyscus maniculatus*, and (*iii*) SNV passaged five times in Vero E6 cells, no amino acid sequence differences were observed in any protein (45). NY-1 and SNV hantaviruses were used at similar low Vero E6

passages (6–7) in these studies, and both viruses were inhibited nearly identically by vitronectin and anti-integrin antibodies.

HPS results in severe pulmonary edema along with a noted increase in capillary permeability in patients (3, 5). Endothelial cells, line the vasculature and maintain vascular integrity through cellular adherence which is partly conferred by $\alpha_v\beta_3$ integrins. In fact, transcapillary fluid fluxes involved in pulmonary edema are directly regulated by $\alpha_v\beta_3$ integrins on pulmonary endothelial cells (14, 20). As a result, hantavirus interactions with $\alpha_v\beta_3$ integrins provide a clear means for altering vascular permeability during infection and a potential point for therapeutic intervention during hantavirus infection.

This report indicates that virus infects $\alpha_{IIb}\beta_3$-expressing cells. $\alpha_{IIb}\beta_3$ is the predominant adhesion receptor present on platelets, and pathogenic hantaviruses cause acute platelet loss, which could be directly related to the use of $\alpha_{IIb}\beta_3$ integrins during infection. Hantaviruses could function similarly to the 7E3 antibody (Fab:ReoPro), which recognizes both $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ integrins and inhibits platelet aggregation (46, 47). Hantaviruses also may use platelets for transport to new infection sites although it is not known whether hantaviruses damage, enter, or replicate within platelets. However, this provides a potential mechanism for hantavirus regulation of platelet function, through altered platelet signaling (35, 48, 49). Given the abundance of platelet $\alpha_{IIb}\beta_3$ receptors, intracellular regulation is a more plausible mechanism for altering platelet function than is a direct hantavirus-receptor blockade.

Although integrins are used by a few icosahedral viruses this report demonstrates that enveloped viruses also use integrins for cellular entry. Hantaviruses are enveloped viruses although they have a highly ordered grid-like surface structure which surrounds and is anchored to the viral envelope (1). It is possible that this unique structure requires the participation of integrins for hantavirus entry into cells. However, it is just as plausible that hantaviruses are the first of many enveloped viruses, which will be found to enter cells through interactions with integrins.

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1. Schmaljohn, C. & Patterson, J. (1990) in *Virology*, ed. Fields, B. (Raven, New York), Vol. 1, pp. 1175–1190.
2. Nichol, S., Spiropoulou, C., Morzunov, S., Rollin, P., Ksiazek, T., Feldmann, H., Sanchez, A., Childs, J., Zaki, S. & Peters, C. J. (1993) *Science* **262,** 914–917.
3. Nolte, K., Feddersen, R., Foucar, K., Zaki, S. Koster, F., Madar, D., Merlin, T., McFeeley, P., Umland, E. & Zumwalt, R. (1995) *Hum. Pathol.* **26,** 110–120.
4. Hughes, J., Peters, C. J., Cohen, M. & Mahy, B. (1993) *Science* **262,** 850–851.
5. Zaki, S., Greer, P., Coffield, L., Goldsmith, C., Nolte, K., Foucar, K., Feddersen, R., Zumwalt, R., Miller, G., Rollin, P., *et al.* (1995) *Am J. Pathol.* **146,** 552–579.
6. Khan, A., Khabbaz, R., Armstrong, L., Holman, R., Bauer, S., Graber, J., Strine, T., Miller, G., Reef, S., Tappero, J., *et al.* (1996) *J. Infect. Dis.* **173,** 1297–1303.
7. Lopez, N., Padula, P., Rossi, C., Lazaro, M. & Franze-Fernandez, M. (1996) *Virology* **220,** 223–226.
8. Hjelle, B., Torrez-Martinez, N. & Koster, F. T. (1996) *Lancet* **347,** 57.
9. Levis, S., Rowe, J., Morzunov, S., Enria, D. & St. Jeor, S. (1997) *Lancet* **349,** 998–999.
10. Yanagihara, R. & Silverman, D. (1990) *Arch. Virol.* **111,** 281–286.
11. Pensiero, M., Sharefkin, J., Dieffenbach, C. & Hay, J. (1992) *J. Virol.* **66,** 5929–5936.
12. Butcher, E. & Picker, L. (1996) *Science* **272,** 60–66.
13. Lampugnani, M., Resnati, M., Dejana, E. & Marchisiio, P. (1991) *J. Cell Biol.* **112,** 479–490.
14. Hawiger, J. (1995) *Thromb. Haemostasis* **74,** 369–372.
15. Cheresh, D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6471–6475.
16. Sepp, N., Li, L. J., Lee, K. H., Brown, E., Caughman, S., Lawley, T. & Swerlick, R. (1994) *J. Invest. Dermatol.* **103,** 295–299.
17. Phillips, D., Charo, I. & Parise, L. (1988) *Blood* **71,** 831–843.
18. Hynes, R. (1992) *Cell* **69,** 11–25.
19. Sugimori, T., Griffith, D. & Arnaout, M. (1997) *Kidney Int.* **51,** 1454–1462.
20. Tsukada, H., Ying, X., Fu, C., Ishikawa, S., McKeown-Longo, P., Albelda, S., Bhattacharya, S., Bray, B. & Bhattacharya, J. (1995) *Circ. Res.* **77,** 651–659.
21. Diaz-Gonzalez, F., Forsyth, J., Steiner, B. & Ginsberg, M. (1996) *Mol. Biol. Cell* **7,** 1939–1951.
22. Loftus, J., O'Toole, T., Plow, E., Glass, A., Frelinger, A., III & Ginsberg, M. (1990) *Science* **249,** 915–918.
23. Hjelle, B., Lee, S., Song, W., Torrez-Martinez, N., Song, J. W., Yanagihara, R., Gavrilovskaya, I. & Mackow, E. (1995) *J. Virol.* **69,** 8137–8141.
24. Schmaljohn, A., Li, D., Negley, D., Bressler, D. S., Turell, M., Korch, G., Ascher, M. & Schmaljohn, C. (1995) *Virology* **206,** 963–972.
25. Yanagihara, R., Daum, C. A., Lee, P. W., Baek, L., Amyx, H., Gajdusek, D. & Gibbs, C. (1987) *Trans. R. Soc. Trop. Med. Hyg.* **81,** 42–45.
26. Song, J., Baek. L., Gavrilovskaya, I., Mackow, E., Hjelle, B. & Yanigahara, R. (1996) *Virus Genes* **12,** 249–256.
27. Shaw, R., Vo, P., Offit, P., Coulson, B. & Greenberg, H. (1986) *Virology* **155,** 434–451.
28. Charo, I., Nannizzi, L., Smith, J. & Cheresh, D. (1990) *J. Cell Biol.* **111,** 2795–2800.
29. D'Souza, S., Ginsberg, M., Burke, T., Lam, S. & Plow, E. (1988) *Science* **242,** 91–93.
30. Smith, J. & Cheresh, D. (1988) *J. Biol. Chem.* **263,** 18726–18731.
31. Loftus, J., Smith, J. & Ginsberg, M. (1994) *J. Biol. Chem.* **269,** 25235–25238.
32. Faull, R. & Ginsberg, M. (1996) *J. Am. Soc. Nephrol.* **7,** 1091–1097.
33. Huttenlocher, A., Ginsberg, M. & Horwitz, A. (1996) *J. Cell Biol.* **134,** 1551–1562.
34. Ylanne, J., Huuskonen, J., O'Toole, T. E., Ginsberg, M., Virtanen, I. & Gahmberg, C. (1995) *J. Biol. Chem.* **270,** 9550–9557.
35. Schwartz, M., Shaller, M. & Ginsberg, M. (1995) *Annu. Rev. Cell Dev. Biol.* **11,** 549–599.
36. Huang, S., Kamata, T., Takada, Y., Ruggeri, Z. & Nemerow, G. (1996) *J. Virol.* **70,** 4502–4508.
37. Wickham, T., Mathias, P., Cheresh, D. & Nemerow, G. (1993) *Cell* **73,** 309–319.
38. Evander, M., Frazer, I., Payne, E., Qi, Y., Hengst, K. & McMillan, N. (1997) *J. Virol.* **71,** 2449–2456.
39. Coulson, B., Londrigan, S. & Lee, D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 5389–5394.
40. Berinstein, A., Roivainen, M., Hovi, T., Mason, P. & Baxt, B. (1995) *J. Virol.* **69,** 2664–2666.
41. Roivainen, M., Piirainen, L., Hovi, T., Virtanen, I., Riikonen, T., Heino, J. & Hyypia, T. (1994) *Virol.* **203,** 357–365.
42. Bergelson, J., Shepley, M., Chan, B., Hemler, M. & Finberg, R. (1992) *Science* **255,** 1718–1720.
43. Bergelson, J., Chan, B., Finberg, R. & Hemler, M. (1993) *J. Clin. Invest.* **92,** 232–239.
44. Greber, U., Willetts, M., Webster, P. & Helenius, A. (1993) *Cell* **75,** 477–486.
45. Chizhikov, V., Spiropoulou, C., Morzunov, S., Monroe, M., Peters, C. & Nichol, S. (1995) *J. Virol.* **69,** 8132–8136.
46. Reverter, J., Beguin, S., Kessels, H., Kumar, R., Hemler, H. & Coller, B. (1996) *J. Clin. Invest.* **98,** 863–874.
47. Coller, B. (1997) *J. Clin. Invest.* **99,** 1467–1471.
48. Clark, E. A. & Brugge, J. (1995) *Science* **268,** 233–239.
49. Negrescu, E. & Siess, W. (1996) *J. Biol. Chem.* **271,** 26547–26553.