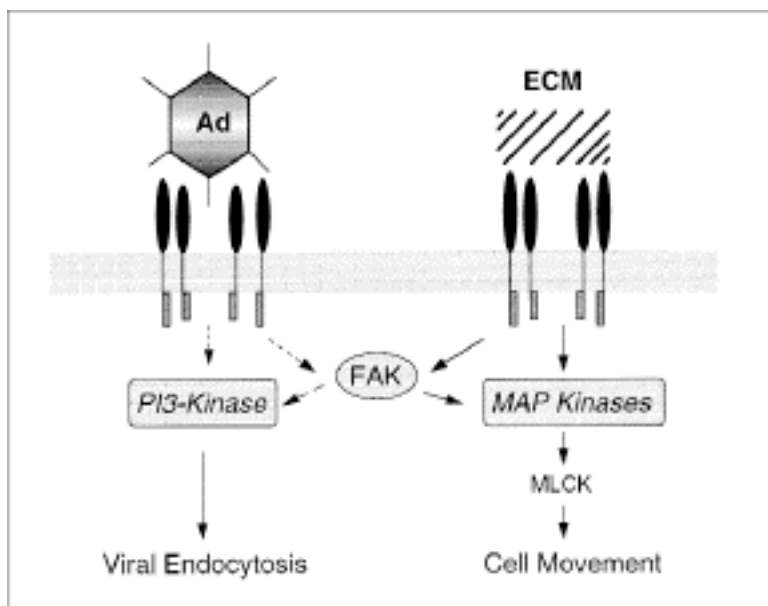




1997

## Integrin-Mediated Signaling Events Involved in Adenovirus Internalization and Cell Motility

E. Li, D.G. Stupack, R. Klemke, D.A. Cheresh, G.R. Nemerow



**Fig. 1.**  $\alpha_v$ -Mediated signaling pathways. Ad = adenovirus, ECM = extracellular matrix, PI3 = phosphatidylinositol-3, FAK = focal adhesion kinase.

Integrin-mediated signaling events mediate a variety of diverse cellular functions, including adhesion, motility, and cell growth. Integrins have also been co-opted by a number of human viruses to allow infection of susceptible host cells. However, the precise mechanisms underlying integrin-mediated viral infection are poorly understood.

Previous studies in our laboratory showed that human adenoviruses use integrins  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  to promote internalization. In recent studies, we investigated the mechanisms by which  $\alpha_v$  integrins promote viral uptake by cells. We found that several signaling molecules, including focal adhesion kinase and phosphatidylinositol-3-kinase, become phosphorylated during interaction of the adenovirus penton base with  $\alpha_v$  integrins. Kinase phosphorylation is also associated with increased activation of phosphatidylinositol-3-kinase. Pharmacologic agents and genetic dysregulation of phosphatidylinositol-3-kinase blocked internalization of adenovirus and infection yet had no effect on integrin-mediated cell adhesion or motility, a cellular process regulated by the Erk1/Erk2 MAP kinase pathway. These studies indicate that integrin ligation by the extracellular matrix or by viral proteins can lead to the engagement of distinct signaling pathways that promote viral endocytosis or cell movement (Fig. 1).

## Retargeting of Recombinant Adenoviral Vectors for Gene Therapy

D.J. Von Seggern, J. Kehler, R. Endo, G.R. Nemerow

Adenoviral vectors are currently being used in a number of clinical trials to deliver therapeutic genes to a variety of cell and tissue types. Unfortunately, several obstacles to the widespread clinical use of adenoviral vectors remain, including the host immune response to virally encoded proteins and the lack of selectivity in tissue targeting. We are attempting to address some of these problems through the development of modified viral vectors.

We have developed mammalian cell lines that stably express the adenovirus type 5 fiber protein in the fiber's native trimeric form. This protein mediates viral attachment to a 46-kD receptor that is expressed on human epithelial cells. The packaging cells can complement a temperature-sensitive mutant adenovirus type 5 that lacks a functional fiber protein and allow incorporation of the adenovirus type 5 fiber protein into viral particles of a different serotype. Further studies are in progress to determine whether a

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

replication-defective adenovirus lacking the fiber gene can be propagated in the fiber-packaging cell lines and to determine whether fiber proteins with different receptor specificities can be incorporated into modified viral vectors.

---

## Inhibition of a Latent Viral Infection and Cell Proliferation by Adenovirus-Delivered Ribozymes

**S. Huang, D.G. Stupack, P. Mathias, Y. Wang, G.R. Nemerow**

Epstein-Barr virus (EBV), a member of the herpesvirus family, persists in latently infected B cells for the life of the host. Under certain conditions, infection is associated with the development of several human malignant tumors and lymphoproliferative diseases in immunocompromised patients such as those with AIDS or in transplant recipients. Currently, little, if any, effective treatment is available for EBV-induced lymphoproliferative diseases. As an approach for treating EBV infections, we examined the capacity of recombinant adenoviral vectors to deliver antiviral ribozymes directed against the EBV nuclear antigen 1 (EBNA-1). EBNA-1 is uniquely expressed in all latently infected B cells and plays an essential role in persistence of the EBV genome. EBNA-1 also reportedly has oncogenic potential.

In contrast to normal B lymphocytes, EBV-transformed B lymphoblastoid cells expressed  $\alpha_v$  integrins and were also susceptible to adenovirus-mediated gene delivery. Adenovirus delivery of an EBNA-1 specific ribozyme to lymphoblastoid cell lines suppressed EBNA-1 mRNA and protein expression. The ribozyme also significantly reduced the number of EBV genomes in latently infected B cells and greatly inhibited B-cell proliferation in medium containing low concentrations of serum.

In recent studies to investigate the use of adenovirus-delivered ribozymes in preventing EBV-induced lymphoproliferative disease in vivo, we used a model system in which mice with severe combined immunodeficiency are reconstituted with human cells. Pretreatment of EBV-transformed B cells with adenovirus-encoded EBNA-1 ribozymes delayed the outgrowth of B-cell tumors and reduced tumor mass in these mice. These studies indicate the potential use of adenovirus-encoded ribozymes in the treatment of EBV-induced lymphoproliferative disorders. Adenoviral vectors are also valuable tools for investigating the precise events in EBV-induced B-cell transformation.

### PUBLICATIONS

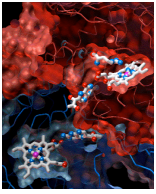
**Huang, S., Jiang, Y., Li, Z., Mathias, P., Lin, S., Ulevitch, R.J., Nemerow, G.R., Han, J.** Apoptosis signaling pathways in T cells is composed of ICE/Ced-3 family proteases and MAP kinase 6b. *Immunity* 6:739, 1997.

**Huang, S., Stupack, D., Mathias, P., Wang, Y., Nemerow, G.** Growth arrest of Epstein-Barr virus immortalized B lymphocytes by adenovirus delivered ribozymes. *Proc. Natl. Acad. Sci. U.S.A.* 94:8156, 1997.

**Stewart, P., Chiu, C., Huang, S., Muir, T., Zhao, Y., Chait, B., Mathias, P., Nemerow, G.R.** Cryo-EM visualization of an exposed RGD epitope on adenovirus that escapes antibody neutralization. *EMBO J.* 16:1189, 1997.

**Stewart, P.L., Nemerow, G.R.** Recent structural solutions for antibody neutralization of viruses. *Trends Microbiol.* 5:22, 1997.

**Von Seggern, D.J., Nemerow, G.R.** Adenoviral vectors for protein expression. *In: Nature: The Palette for the Art of Expression.* Fernandez, J., Hoeffler, J. (Eds.). Academic Press, New York, *in press*.



## 1998

---

### Targeted Adenovirus-Based Vectors in Gene Therapy

**D.J. Von Seggern, S.K. Fleck, M.A. Friedlander, P. Ghazal, G.R. Nemerow**

Targeted gene delivery is a major goal for treating inherited and chronic diseases. Adenovirus-based vectors are currently being used in preclinical and clinical trials for gene therapy. However, these vectors cannot yet be targeted to desired cell types *in vivo* because the cell receptor recognized by the fiber protein of adenovirus type 5 is widely distributed. We have generated an adenovirus type 5 vector that lacks the gene for the fiber protein; this vector can be propagated in a packaging cell line that expresses the wild-type fiber protein. Cell lines are also being generated that express a fiber protein derived from a different viral serotype (adenovirus type 3) that confers a distinct cell-binding specificity. Genetic modifications of the fiber protein of adenovirus type 5 are also in progress to abrogate attachment to the cell receptor for the protein and to insert epitopes that provide a new cell-binding specificity.

Studies have also been started as part of a recently established ocular gene therapy program to treat inherited or acquired diseases of the eye involving macular degeneration. Adenovirus vectors will be used to deliver a normal *rds/peripherin* gene to murine photoreceptors in an attempt to prevent macular degeneration and loss of visual function in mice deficient in *rds/peripherin*. Tissue-specific or regulatable promoters will be used to optimize transgene expression.

---

### Structural Analysis of $\alpha_v$ Integrin Interactions With Human Adenoviruses

**P. Mathias, C. Chiu,\* P.L. Stewart,\* G.R. Nemerow**

*\* University of California, Los Angeles, CA*

Although  $\alpha_v$  integrins serve as coreceptors for entry of adenovirus into cells, little is known of the precise interactions of these molecules with host cell-derived or viral ligands. To gain further insights into the structure and function of  $\alpha_v$  integrins, we have produced a soluble form of the integrin  $\alpha_v\beta_5$  that retains the ability to bind vitronectin as well as multiple adenovirus serotypes. Using cryoelectron microscopy and image reconstruction, we have analyzed the binding of soluble integrin molecules to different adenovirus serotypes. Integrins were visualized bound to adenovirus type 12, a serotype containing a relatively short (19 amino acid) arginine-glycine-aspartic acid domain. These structural studies also revealed the site of integrin binding and intermolecular associations of bound receptors. Further studies are in progress to characterize the kinetics and stoichiometry of integrin binding to different adenovirus serotypes to gain a more complete understanding of integrin associations with viral ligands.

---

### Regulation of $\alpha_v$ Integrin Expression on B Lymphocytes Transformed by Epstein-Barr Virus

**S. Huang, D.G. Stupack, G.R. Nemerow**

Although  $\alpha_v$  integrins have an important role in several biological processes, comparatively little is known of how integrin expression is regulated in specific cell types. Infection of human B lymphocytes by Epstein-Barr virus (EBV) upregulates expression of  $\alpha_v$  integrins. Experiments have revealed that the EBV-encoded latent gene products LMP-1 and EBNA-2 promote activation of the  $\alpha_v$  integrin promoter in EBV-transformed B cells. Studies are under way to define the signaling pathways that lead to activation of the

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

promoter and to determine the transcription factors and DNA promoter sequences that regulate integrin expression. These studies should provide further insights into the control of  $\alpha_v$  integrin expression and also of the viral and host cell factors involved in EBV-induced B-cell metastasis.

---

## Integrin-Mediated Signaling Events Involved in Adenovirus Endocytosis

**E. Li, D.G. Stupack, K. Wang, D.A. Cheresh, G.M. Bokoch, G.R. Nemerow**

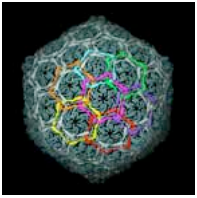
Adenovirus type 2 particles enter cells via clathrin-mediated endocytosis. Internalization of virus via  $\alpha_v$  integrins also requires activation of phosphatidylinoside-3-OH kinase. In recent studies, we showed that entry of virus is associated with reorganization of the actin cytoskeleton. Viral particles induce polymerization of cortical actin filaments and formation of membrane ruffles and microspikes at the cell surface, processes that require activation of phosphatidylinoside-3-OH kinase. Entry of virus is also blocked by treatment of cells with cytochalasin D, indicating that an intact actin cytoskeleton is required for internalization of virus. In further studies, we showed that the Rho family of small GTPases, Rac and Cdc42, act downstream of the kinase to promote actin assembly and viral entry. Studies are in progress to determine the upstream signaling molecules and the downstream effector proteins that mediate adenovirus internalization.

### PUBLICATIONS

**Li, E., Stupack, D.G., Klemke, R., Cheresh, D.A.** Adenovirus endocytosis via  $\alpha_v$  integrins requires phosphatidylinoside-3-OH kinase. *J. Virol.* 72:2055, 1998.

**Von Seggern, D., Kehler, J., Endo, R.J., Nemerow, G.R.** Complementation of a fibre mutant adenovirus by packaging cell lines stably expressing the adenovirus type 5 fibre protein. *J. Gen. Virol.* 79:1461, 1998.

**Wang, K., Huang, S., Kapoor-Munshi, A., Nemerow, G.R.** Adenovirus internalization and infection requires dynamin. *J. Virol.* 72:3455, 1998.



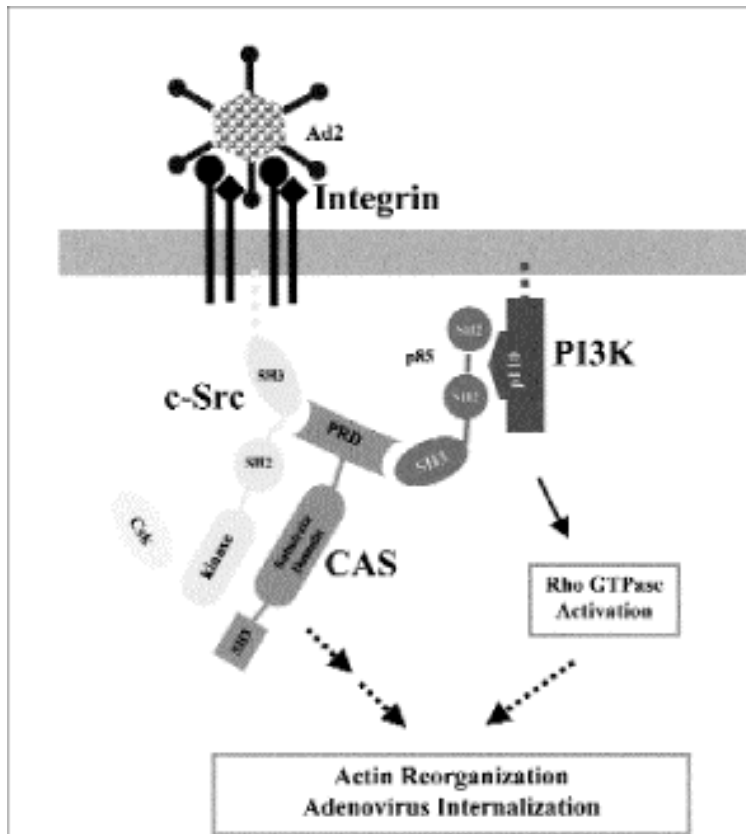
## 1999

### Structure and Kinetic Analyses of Integrin $\alpha_v\beta_5$ Association With Human Adenoviruses

P.L. Stewart, C.Y. Chiu, P. Mathias, G.R. Nemerow

The  $\alpha_v$  integrins are used by human adenoviruses to gain entrance to susceptible host cells. To better understand the molecular basis by which integrins promote internalization of virus, we produced soluble recombinant  $\alpha_v\beta_5$  for kinetic and structural studies. The purified soluble integrin retained the ability to bind both its natural ligand, vitronectin, and the adenovirus penton base protein.

The 3-dimensional structure of integrin  $\alpha_v\beta_5$  bound to human adenoviruses types 2 and 12 was determined at approximately 20-Å resolution by electron cryo-microscopy. The results showed that the integrin consists of 2 discrete subdomains, a globular domain with an arginine-glycine-aspartic acid binding cleft approximately 20 Å in diameter and a distal domain with extended flexible tails. Kinetic analysis, done with an automated biosensor, indicated that 4-5 integrins bind to each penton base protein on the virus particle. These studies suggest that the precise spatial arrangement of the 5 arginine-glycine-aspartic acid sites on the penton base protein promotes integrin clustering and the signaling events required for internalization of virus.



### Integrin-Mediated Signaling Events in Adenovirus Internalization

E. Li, D. Stupack, S.L. Brown, R. Klemke, D. Schlaepfer, D. Cheresh, G.R. Nemerow

Internalization of adenovirus via  $\alpha_v$  integrins also requires activation of a lipid kinase, phosphatidylinositol-3'-kinase, and Rho family GTPases. These signaling molecules mediate reorganization of the actin cytoskeleton, an event required for efficient adenovirus internalization. In a continuation of these studies, we found that internalization of adenovirus also involves the signaling intermediate known as p130<sup>Cas</sup> (Crk-associated substrate, CAS). Entry of virus into cells requires phosphorylation of CAS. Expression of dominant-negative forms of CAS but not FAK, another signaling molecule involved in integrin-mediated cell adhesion, also blocks adenovirus entry. We found that CAS associates with the SH3 domain of p85/phosphatidylinositol-3'-kinase via a 3 amino acid motif (RXL) in the proline-rich region of CAS that contains arginine and leucine plus any other amino acid (Fig. 1). These studies reveal the molecular basis by which CAS coordinates the distinct signaling pathways involved in integrin-mediated viral endocytosis and cell adhesion.

**Fig. 1.** Schematic diagram of the signaling molecules involved in adenovirus internalization mediated by  $\alpha_v$  integrin.

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

---

## Characterization of a Novel Cell Receptor for Subgroup D Adenoviruses That Cause Severe Ocular Infections

S. Huang, V. Reddy, N. Dasgupta, E. Wu, J. Fernandez, G.R. Nemerow

A 46-kD cell-surface coxsackievirus-adenovirus receptor mediates attachment of adenovirus to cells via interaction with the fiber protein of the virus. Although many adenovirus serotypes use this receptor for cell attachment, certain serotypes such as adenovirus 37, a virus associated with epidemic keratoconjunctivitis, use a separate receptor to infect human conjunctival cells. We found that a single amino acid residue (Lys240) located at the apex of an extended loop in the fiber knob domain of adenovirus 37 confers binding to conjunctival cells. A closely related virus, adenovirus 19p, which contains a glutamic acid at position 240 in the fiber protein, does not bind and infect conjunctival cells. Replacement of the glutamic acid by lysine in adenovirus 19p conferred cell binding of the virus, whereas replacement of the lysine with glutamic acid in adenovirus 37 abrogated cell binding. Further studies are in progress to characterize the receptor for adenovirus 37 on human conjunctival cells.

---

## Pseudotyping Adenovirus Vectors to Enhance Virus-Mediated Gene Delivery

D.J. Von Seggern, S. Huang, S.K. Fleck, S.C. Stevenson, J. Corbin, G.R. Nemerow

Although gene therapy vectors based on adenovirus type 5 have a broad cell tropism, not all cells can be transduced by this type of vector. In particular, cells of hematopoietic origin are resistant to adenovirus infection. Retargeting adenovirus type 5 vectors to achieve a broader or more specific gene delivery is a goal of several laboratories. We recently developed a versatile system for retargeting adenovirus vectors that uses a fiber-deleted vector, designated Ad5 $\delta$ F, and packaging cells that express a different fiber protein. Using this system, we generated Ad5 $\delta$ F particles equipped with an adenovirus type 3 fiber protein that recognizes a host cell receptor distinct from the receptor used by adenovirus type 5. The adenovirus type 3 pseudotyped virions infected B-lymphoblastoid cells at least 10-fold more efficiently than did adenovirus type 5 vectors. These studies provide a proof that pseudotyping can increase the host range of adenovirus gene-delivery vectors.

### PUBLICATIONS

**Chiu, C.Y., Mathias, P., Nemerow, G.R., Stewart, P.L.** Structure of adenovirus complexed with its internalization receptor,  $\alpha_v\beta_5$  integrin. *J. Virol.* 73:6759, 1999.

**Huang, S., Reddy, V., Dasgupta, N., Nemerow, G.R.** A single amino acid in the adenovirus type 37 fiber confers binding to human conjunctival cells. *J. Virol.* 73:2798, 1999.

**Li E., Stupack, D., Bokoch, G.M., Nemerow, G.R.** Adenovirus endocytosis requires actin cytoskeleton reorganization mediated by Rho family GTPases. *J. Virol.* 72:8806, 1998.

**Mathias, P., Galleno, M., Nemerow, G.R.** Interactions of soluble recombinant integrin  $\alpha_v\beta_5$  with human adenoviruses. *J. Virol.* 72:8669, 1998.

**Nemerow, G.R., Stewart, P.L.** The role of  $\alpha_v$  integrins in adenovirus cell entry and gene delivery. *In: Microbiology and Molecular Biology Reviews.* Keene, J.D. (Ed.). American Society of Microbiology Publications, Washington, DC, *in press*.

**Pampori, N., Hato, T., Stupack, D.G., Aidoudi, S., Cheresch, D.A., Nemerow, G.R., Shattil, S.J.** Mechanisms and consequences of affinity modulation of integrin  $\alpha_v\beta_3$  detected with a novel patch-engineered monovalent ligand. *J. Biol. Chem.* 274:21609, 1999.

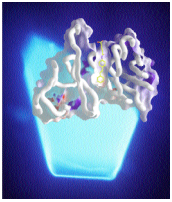
# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

**Stupack, D.G., Li, E., Silletti, S.A., Kehler, J.A., Geahlen, R.L., Hahn, K., Nemerow, G.R., Cheresch, D.A.** Matrix valency regulates integrin-mediated lymphoid adhesion via Syk kinase. *J. Cell Biol.* 144:777, 1999.

**Von Seggern, D., Chiu, C.Y., Fleck, S.K., Stewart, P.L., Nemerow, G.R.** A helper-independent adenovirus vector with E1, E3 and fiber deleted: Structure and infectivity of fiberless particles. *J. Virol.* 73:1601, 1999.

**Von Seggern, D.J., Nemerow, G.R.** Adenoviral vectors for protein expression. *In: Gene Expression Systems: Using Nature for the Art of Expression.* Fernandez, J., Hoeffler, J. (Eds.). Academic Press, San Diego, 1999, p. 111.



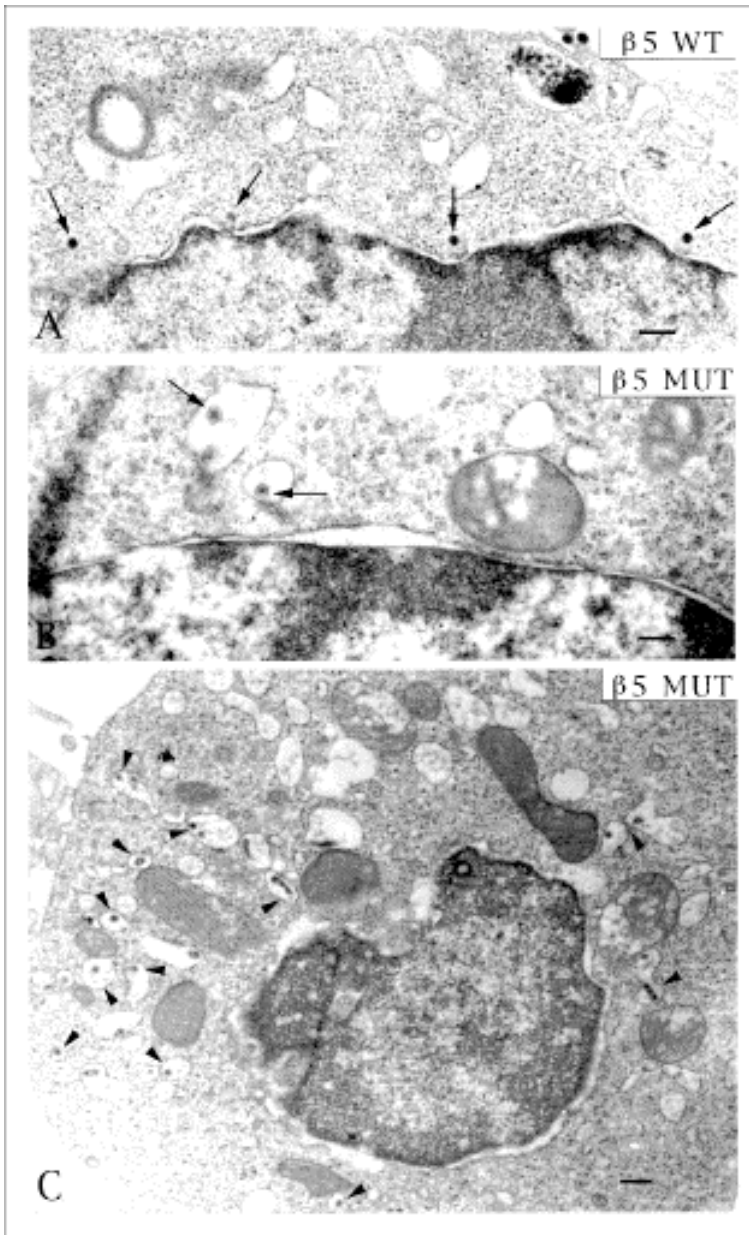
2000

## The Role of $\alpha_v$ Integrins and Signaling Events Required for Adenovirus Cell Entry

E. Li, K. Wang, R. Klemke, D. Stupack, D. Schlaepfer, S.L. Brown, T. Guan, D. Cheresch, G.R. Nemerow

Internalization of adenovirus by cells requires interaction of the penton base capsid protein of the virus with the integrins  $\alpha_v\beta_3$  or  $\alpha_v\beta_5$ . Integrin clustering induces signaling events and subsequent activation of phosphatidylinositol-3-OH kinase, Rho family GTPases, and p130<sup>CAS</sup>. A major downstream target of this signaling pathway is the actin cytoskeleton. Reorganization of cortical actin filaments via integrin signaling is required for efficient internalization of virus.

In addition to its role in adenovirus internalization, integrin  $\alpha_v\beta_5$  also facilitates adenovirus-mediated disruption of endosomes, a process required for viral entry into the cytoplasm and transport to the nucleus. In recent studies, we found that the cytoplasmic tail of the  $\beta_5$  integrin regulates adenovirus-mediated membrane permeabilization and endosome disruption. CS-1 melanoma cells expressing a wild-type  $\beta_5$  integrin supported efficient infection by adenovirus, whereas cells expressing chimeric integrin molecules composed of the  $\beta_5$  ectodomain/transmembrane domains and a  $\beta_3$  cytoplasmic tail had decreased infection. Chimeric  $\beta_5/\beta_3$  integrins facilitated internalization of virus but did not support efficient viral penetration from the endosome (Fig. 1). Further mutagenesis studies revealed that the C-terminal threonine--valine--aspartic acid motif in the  $\beta_5$  integrin cytoplasmic domain plays a major role in regulating adenovirus penetration. Ongoing studies focus on determining the precise role of the  $\beta_5$  integrin cytoplasmic tail in adenovirus cell entry.



**Fig. 1.** Electron micrographs of adenovirus entry into CS-1 cells expressing wild-type ( $\beta_5$  WT)  $\beta_5$  integrin (A) or truncated ( $\beta_5$  MUT)  $\beta_5$  integrin (B, C). Adenovirus particles (arrows and arrowheads) are located at the nuclear membrane in the wild-type cells, whereas they do not escape endosomes in  $\beta_5$  MUT cells.



# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

---

## Characterization of a Receptor for Adenoviruses Associated With Severe Ocular Infections

**E. Wu, S. Huang, D.J. Von Seggern, J. Fernandez, S.K. Fleck, G.R. Nemerow**

Human adenoviruses cause respiratory, gastrointestinal, and ocular infections. A 46-kD membrane protein, coxsackievirus-adenovirus receptor, acts as the receptor for most but not all adenovirus serotypes. Unfortunately, the identification of this receptor as the primary receptor for adenoviruses does not explain why certain serotypes of adenovirus (i.e., types 8, 19, and 37) are highly associated with severe ocular infections, such as epidemic keratoconjunctivitis.

On the basis of our previous studies, we hypothesized that adenoviruses associated with epidemic keratoconjunctivitis recognize a cell receptor that is distinct from coxsackievirus-adenovirus receptor. Consistent with this hypothesis, we found that adenovirus type 37 binds efficiently to Chang C conjunctival cells but not to A549 lung epithelial cells. Both of these cell types express high levels of coxsackievirus-adenovirus receptor. Binding of adenovirus type 37 to the conjunctival cells was calcium dependent and was not inhibited by preincubation with a function-blocking monoclonal antibody to coxsackievirus-adenovirus receptor.

Using a virus overlay protein blot assay, we detected specific binding of adenovirus type 37 particles to a 50-kD protein that is expressed on Chang C conjunctival cells but not on A549 lung epithelial cells. Adenovirus type 19p, a closely related serotype that is nonpathogenic, did not recognize the 50-kD protein. Further biochemical analyses are under way to identify the 50-kD protein and determine its role in adenovirus-mediated epidemic keratoconjunctivitis.

---

## Regulation of Tumor Cell Growth and Invasion by $\alpha_v$ Integrins and Cell-Associated Proteases

**S. Huang, J. Han, D. Stupack, A. Liu, D. Cheresch, G.R. Nemerow**

Although many cell types express  $\alpha_v$  integrins and are susceptible to infection by adenovirus, human B lymphocytes do not express these integrins and are therefore resistant to infection and gene delivery by adenovirus. In contrast, infection of B cells by Epstein-Barr virus upregulates integrin expression and makes these cells susceptible to adenovirus infection. In recent studies, we found that the proteins encoded by Epstein-Barr virus, including LMP1, LMP2A, and EBNA2, selectively upregulate the  $\alpha_v$  integrin promoter. As a consequence of increased expression of  $\alpha_v$  integrins, B cells transformed by Epstein-Barr virus have increased growth capacity in low concentrations of serum and enhanced invasive properties. The latter property is associated with increased expression of matrix metalloproteinase 9. These studies provide further evidence for the role of  $\alpha_v$  integrins and cell proteases in tumor development.

Overexpression of other cell proteases also plays a key role in tumor formation. In recent studies, we showed that expression of u-plasminogen activator and its receptor on breast cancer cells is regulated by the p38 $\alpha$  MAP kinase pathway. We found that p38 $\alpha$  MAP kinase but not p38 $\beta$  MAP kinase stabilizes mRNA for u-plasminogen activator and its receptor, thereby increasing the half-life and expression of these proteins on breast cancer cells. These studies suggest that compounds directed to specific MAP kinases may have therapeutic potential in the treatment of neoplasia.

### PUBLICATIONS

**Huang, S., New, L., Pan, Z., Han, J., Nemerow, G.R.** Urokinase plasminogen activator/urokinase-specific surface receptor expression and matrix invasion by breast cancer cells requires constitutive p38 $\alpha$  mitogen-activated protein kinase activity. *J. Biol. Chem.* 275:12266, 2000.

**Huang, S., Stupack, D., Liu, A., Cheresch, D., Nemerow, G.R.** Cell growth and matrix invasion of EBV-immortalized human B lymphocytes is regulated by expression of  $\alpha_v$  integrins. *Oncogene* 19:1915, 2000.

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

**Li, E., Stupack, D.G., Brown, S.L., Klemke, R., Schlaepfer, D.D., Nemerow, G.R.** Association of p130<sup>CAS</sup> with phosphatidylinositol-3-OH kinase mediates adenovirus cell entry. *J. Biol. Chem.* 275:14729, 2000.

**Nemerow, G.R.** Recent structure analyses offer new insights for retargeting adenovirus vectors. *Trends Microbiol.*, *in press*.

**Nemerow, G.R., Stewart, P.L.** The role of  $\alpha_v$  integrins in adenovirus cell entry and gene delivery. *Microbiol. Mol. Biol. Rev.* 63:725, 1999.

**Saphire, A.C.A., Guan, T., Schirmer, E.C., Nemerow, G.R., Gerace, L.** Nuclear import of adenovirus DNA in vitro involves the nuclear protein import pathway and hsc70. *J. Biol. Chem.* 275:4298, 2000.

**Von Seggern, D.J., Huang, S., Fleck, S.K., Stevenson, S.C., Nemerow, G.R.** Adenovirus vector pseudotyping in fiber-expressing cell lines: Improved transduction of Epstein-Barr virus-transformed B cells. *J. Virol.* 74:354, 2000.

**Wang, K., Guan, T., Cheresch, D.A., Nemerow, G.R.** Regulation of adenovirus membrane penetration by the cytoplasmic tail of integrin  $\beta_5$ . *J. Virol.* 74:2731, 2000.

**Xia, Y., Makris, C., Su, B., Li, E., Yang, J., Nemerow, G.R., Karin, M.** MEK kinase 1 is critically required for c-Jun N-terminal kinase activation by proinflammatory stimuli and growth factor-induced cell migration. *Proc. Natl. Acad. Sci. U. S. A.* 97:5243, 2000.



## 2001

### **Role of $\alpha_v\beta_1$ Integrin as an Adenovirus Internalization Receptor**

**E. Li, S.L. Brown, D.G. Stupack, X.S. Puente, D.A. Cheresh, G.R. Nemerow**

Human adenovirus vectors are being investigated for use in gene therapy; however, the precise host-cell factors that mediate virus-mediated gene delivery are not fully understood. The integrins  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  promote internalization of adenovirus by host cells after attachment of the virus particle to the cell via the coxsackievirus-adenovirus receptor. The human embryonic kidney cell line HEK293 is commonly used to propagate replication-defective adenovirus vectors for gene delivery; however, HEK293 cells lack  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ . Nevertheless, these cells express the coxsackievirus-adenovirus receptor and are susceptible to adenovirus infection. We therefore considered the possibility that adenovirus may recognize additional internalization receptors.

We found that function-blocking antibodies directed against  $\alpha_v$  or  $\beta_1$ , but not  $\beta_3$  or  $\beta_5$ , integrin subunits could suppress adenovirus infection of and internalization by HEK293 cells. Moreover, adhesion of HEK293 cells to the adenovirus penton base protein was selectively mediated by integrin  $\alpha_v\beta_1$ , whereas adhesion to fibronectin was governed by either  $\alpha_v\beta_1$  or  $\alpha_5\beta_1$ .

These results illustrate the complexity of the interactions between adenovirus and host cells and may explain why mice that lack the gene for the  $\beta_5$  integrin subunit appear to be susceptible to adenovirus infection. Overexpression of integrin  $\alpha_v\beta_1$  on certain cancer cell types may also facilitate treatment of human tumors by oncolytic adenovirus vectors.

### **Structural Analysis of an Adenovirus Vector With Ocular Tropism**

**C.Y. Chiu, P.L. Stewart, D.J. Von Seggern, E. Wu, G.R. Nemerow**

More than 50 different serotypes of human adenovirus are divided among 6 different subgroups (A–F) on the basis of DNA sequence homology and other biological features. The coxsackievirus-adenovirus receptor (CAR) is the primary receptor for most types of adenovirus. However certain subgroup D adenoviruses (i.e. adenovirus type 37) associated with severe ocular infections use an unidentified 50-kD protein for infection of conjunctival epithelial cells.

To gain an understanding of the structural basis of different receptor interactions, we produced an adenovirus type 5 vector equipped with the fiber protein from adenovirus type 37. This fiber-pseudotyped vector retains ocular cell tropism characteristic of wild-type adenovirus type 37. Using electron cryo-microscopy and image reconstructions, we visualized, for the first time, the complete fiber protein, including the distal knob domain. These studies indicated that the adenovirus type 37 fiber is a short and rigid molecule.

Because the adenovirus type 37 fiber knob retains the conserved CAR-binding amino acid sequences, our structural and modeling studies suggest that the relatively short, inflexible adenovirus type 37 fiber cannot bind to CAR because of steric hindrance from the bulky viral capsid. In addition, interactions between the penton base and  $\alpha_v$  integrins may shield the lateral surfaces of the fiber knob from CAR association. Studies with adenovirus vectors equipped with chimeric and truncated forms of the adenovirus type 5 and type 37 fiber proteins are in progress to test these hypotheses.

## Differential Targeting of Specific Cell Types in the Eye by Fiber-Pseudotyped Adenovirus Vectors

**D.J. Von Seggern, E. Aguilar, K. Kinder, R. Summers, J.C. Armas, S.K. Fleck, S.C. Stevenson, P. Ghazal, G.R. Nemerow, M. Friedlander**

Degenerative diseases of the retina affect many persons worldwide, with devastating effects on quality of life. Loss of vision is often directly due to loss of photoreceptors, the cell type responsible for transducing light into neuronal impulses, and several genes involved in retinopathies have been identified. Adenovirus vectors have been evaluated for the delivery of therapeutic genes in the eye. Experiments with current adenovirus vectors that use the coxsackievirus-adenovirus receptor (e.g., adenovirus type 5) have generally been disappointing because little photoreceptor transduction occurred. We hypothesized that photoreceptors lack the coxsackievirus-adenovirus receptor and therefore investigated whether adenovirus vectors equipped with the fiber protein from adenovirus serotypes that do not use the coxsackievirus-adenovirus receptor (adenoviruses types 3 and 37) might improve ocular gene delivery.

Adenovirus particles equipped with the adenovirus type 5 fiber and injected into the vitreous body in mice primarily transduced the iris and corneal endothelia and the ciliary body. Adenovirus vectors with the adenovirus type 3 fiber protein largely transduced the ciliary body. Neither adenovirus type 5 nor adenovirus type 3 vectors transduced photoreceptors. In contrast, adenovirus vectors bearing the adenovirus type 37 fiber protein efficiently and selectively delivered green fluorescent protein to photoreceptors when administered by either intravitreal or subretinal injections. Future studies will determine if adenovirus type 37--based vectors encoding a therapeutic gene can delay or prevent retinal disease in appropriate murine models.

### PUBLICATIONS

**Chiu, C.Y., Wu, E., Brown, S.L., Von Seggern, D.J., Nemerow, G.R., Stewart, P.L.** Structural analysis of a fiber-pseudotyped adenovirus with ocular tropism suggests differential modes of cell receptor interactions. *J. Virol.* 75:5375, 2001.

**Jakubczak, J.L., Rollence, M.L., Stewart, D.A., Jafari, J.D., Von Seggern, D.J., Nemerow, G.R., Stevenson, S.C., Hallenbeck, P.L.** Adenovirus type 5 viral particles pseudotyped with mutagenized fiber proteins show diminished infectivity of coxsackie B-adenovirus receptor-bearing cells. *J. Virol.* 75:2972, 2001.

**Li, E., Brown, S.L., Dolman, C.S., Brown, G.B., Nemerow, G.R.** Production of functional antibodies generated in a nonlytic insect cell expression system. *Protein Expr. Purif.* 21:121, 2001.

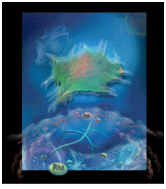
**Li, E., Brown, S.L., Stupack, D.G., Puente, X.S., Cheresch, D.A., Nemerow, G.R.** Integrin  $\alpha_v\beta_1$  is an adenovirus coreceptor. *J. Virol.* 75:5405, 2001.

**Nemerow, G.R.** Adenoviral vectors: New insights. *Trends Microbiol.* 8:391, 2000.

**Nemerow, G.R.** Biology of adenovirus cell entry. *In: Adenoviral Vectors for Gene Therapy.* Curiel, D., Douglas, J. (Eds.). Academic Press, San Diego, *in press*.

**Stewart, P.S., Dermody, T.S., Nemerow, G.R.** Structural basis of nonenveloped virus cell entry. *Adv. Protein Chem., in press.*

**Wu, E., Fernandez, J., Fleck, S.K., Von Seggern, D.J., Huang, S., Nemerow, G.R.** A 50-kDa membrane protein mediates sialic acid-independent binding and infection of conjunctival cells by adenovirus type 37. *Virology* 279:78, 2001.



## 2002

---

### **Structural Features of the Adenovirus Fiber That Regulate Cell-Receptor Interactions**

**L. Pache, E. Wu, D.J. Von Seggern, P.L. Stewart, G.R. Nemerow**

Adenoviruses remain an important pathogen of humans. However, replication-defective or conditionally replicating forms of adenovirus vectors are currently under investigation in about 25% of all human gene therapy trials. A better understanding of adenovirus cell tropism is an important goal in optimizing these viral vectors.

The adenovirus fiber protein, a homotrimeric molecule, mediates viral attachment to host cell receptors. The fiber contains 3 distinct domains: an N-terminal region that associates with the penton base protein, a central shaft domain that contains a variable number of  $\beta$ -helical repeat elements, and a C-terminal knob domain that mediates attachment to host cell receptors. Depending on the virus type, fiber proteins may contain 6 to 23 repeats in the central shaft domain. Interestingly, repeats 3 and 21 of most but not all adenovirus types contain a nonconsensus sequence that may confer fiber flexibility. We hypothesized that the length and/or flexibility of the fiber shaft plays a heretofore unrecognized role in modulating interaction with viral receptors. To test this hypothesis, we created recombinant adenovirus vectors equipped with truncated or chimeric fiber proteins and then tested the ability of the vectors to infect human epithelial cells.

Adenovirus particles equipped with a shortened adenovirus 5 fiber protein lacking 14  $\beta$ -helical repeats in the shaft domain had substantially reduced infectivity. Conversely, lengthening the shaft of the wild-type adenovirus 37 fiber restored infectivity dependent on the coxsackievirus-adenovirus receptor. In further studies, we showed that replacing the flexible  $\beta$ -helical repeats (modules 3 and 21) in the adenovirus 5 fiber with the corresponding repeats from adenovirus 37, which has a rigid fiber protein, abrogated cell infection. Preliminary electron cryomicroscopy structural analyses suggest that this adenovirus 5-adenovirus 37 chimeric fiber protein has decreased flexibility.

These studies indicated that both length and flexibility of the fiber shaft regulate cell-receptor interactions occurring at the distal knob domain. Increased knowledge of the adenovirus fiber structure and its role in receptor interaction should help in the design of viral vectors with increased cell specificity.

---

### **Isolation and Identification of a Cell Receptor for Adenoviruses With Ocular Tropism**

**E. Wu, D.J. Von Seggern, G. Suizdak, G. Nemerow**

A total of 51 different serotypes of human adenoviruses are divided among 6 subgroups (A-F) on the basis of DNA sequence homology and serologic properties. Although many different adenoviruses can cause ocular infections, members of subgroup D, which includes adenovirus serotypes 37, 19a, and 8, are particularly infectious and sometimes cause significant visual disturbances that can last weeks to months. The molecular basis for the ocular tropism of these serotypes is currently poorly understood.

In previous studies, we showed that unlike most adenovirus serotypes, adenovirus 37 does not recognize the coxsackievirus-adenovirus receptor on host cells. This lack of recognition is due in part to the short and rigid fiber protein of adenovirus 37, which, as noted previously, restricts interactions with the receptor. Instead, we showed that adenovirus 37 associates with an unidentified 50-kD membrane protein that is highly expressed on conjunctival epithelial cells. Interestingly, adenovirus 19p, a nonpathogenic virus that is highly homologous to adenovirus 37 does not bind the 50-kD protein. This feature allowed us to identify a key amino acid residue, lysine 240, in the adenovirus 37 knob domain that regulates interaction with the putative cell receptor for the 50-kD protein.

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

We are using lectin affinity chromatography and 2-dimensional gel electrophoresis to isolate the 50-kD protein. Protein spots/bands are excised, digested with trypsin, and subjected to high-performance liquid chromatography and tandem mass spectrometry. Using this approach, we identified 4 candidate membrane proteins. Functional studies are now in progress to determine whether any of these proteins acts as a bonafide receptor for adenovirus 37.

Further studies will determine if multiple members of subgroup D adenoviruses use the same or different cell receptors. The identification of a cell receptor for subgroup D adenoviruses may shed further light on the molecular basis for the ocular tropism and pathogenesis of these serotypes. Because few if any antiviral agents are currently available for treating adenovirus ocular infections, the identification of the adenovirus 37 receptor may also provide an additional target for therapeutic intervention.

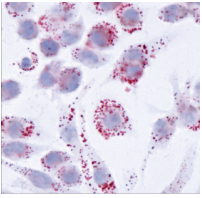
## PUBLICATIONS

**Nemerow, G.R.** Biology of adenovirus cell entry. *In: Adenoviral Vectors for Gene Therapy*. Curiel, D., Douglas, J. (Eds.). Academic Press, San Diego, 2002, p. 19.

**Nemerow, G.R., Cheresch, D.A.** Herpesvirus hijacks an integrin. *Nat. Cell Biol.* 4:E69, 2002.

**Nemerow, G.R., Stewart, P.L.** Antibody neutralization epitopes and integrin binding sites on nonenveloped viruses. *Virology* 288:189, 2001.

**Nicklin, S.A., Von Seggern, D.J., Wok, L.M., Pek, D.C.K., Dominiczak, A.F., Nemerow, G.R., Baker, A.H.** Ablating adenovirus type 5 fiber-CAR and HI loop insertion of the SIGYPLP peptide generates an endothelial cell-selective adenovirus. *Mol. Ther.* 4:534, 2001.



## 2003

---

### Receptors for Adenoviruses Associated With Ocular Pathogenesis

E. Wu, L. Pache, T. Mullen, S. Trauger, G. Siuzdak, G.R. Nemerow

Most, but not all, of the 51 different serotypes of human adenovirus use a 42-kD membrane protein known as coxsackievirus-adenovirus receptor for high-affinity binding to and infection of host cells. However, the broad distribution of this receptor on many different cell types does not readily explain why certain serotypes of adenoviruses have restricted tissue tropism in vivo. In this regard, subgroup D adenoviruses are highly associated with severe ocular infections (epidemic keratoconjunctivitis). Adenovirus type 37, a member of the D subgroup of adenoviruses, recognizes a 50-kD membrane protein on conjunctival cells that is distinct from the coxsackievirus-adenovirus receptor.

Recently, using lentil lectin chromatography, we partially purified the 50-kD membrane protein, and using liquid chromatography coupled to ion-trap tandem mass spectrometry, we identified several candidate receptors. Immunoprecipitation analyses revealed that antibodies directed against CD46 specifically recognized the 50-kD receptor. CD46 (also termed membrane cofactor protein) is an integral membrane protein that protects cells from the complement system. This protein is also a receptor for measles virus, human herpesvirus type 6, and *Neisseria gonorrhoeae*.

Immunofluorescence analysis indicated that CD46 is expressed in the cornea and lens, consistent with its potential role in ocular pathogenesis associated with adenovirus infection. Expression of CD46 on CHO and COS-7 cells in vitro also enhanced adenovirus binding and gene delivery dependent on the fiber of adenovirus type 37, indicating that CD46 can function as a receptor for adenovirus type 37. Further studies are in progress to determine whether CD46 is used as a receptor by other subgroup D adenoviruses (i.e., types 19a and 8) that promote ocular pathogenesis in vivo.

---

### Late Trafficking Events in Cell Entry by Adenoviruses

M. Martinez, C. Wiethoff, G.R. Nemerow

Integrin-mediated internalization of adenovirus by host cells requires signaling molecules, including phosphatidylinositol-3'-kinase, the Rho family of small GTPases, and p130CAS. This signaling pathway promotes actin polymerization, a process needed for efficient internalization of the virus. Interestingly, late trafficking events in entry of adenoviruses into host cells also appear to require signaling processes. Thus, treatment of cells with calphostin C, an inhibitor of protein kinase C (PKC) blocks adenovirus-mediated gene delivery. Binding or internalization of adenoviruses is not altered by calphostin C, suggesting that PKC is involved in a late step in viral entry, such as endosome trafficking or endosome disruption. Consistent with this possibility, adenovirus particles accumulate inside intracellular vesicles in the periphery of cells treated with calphostin C.

Our preliminary findings suggest that an atypical PKC isoform specifically regulates adenovirus cell entry, because calphostin C inhibition of adenovirus infection can be partially overcome by overexpressing a wild type of the isoform. In further studies, we will attempt to confirm these findings to determine the precise role of the PKC isoform in adenovirus entry. These investigations may also shed light on the normal physiologic role of atypical PKC isoforms.

In general, viruses must be sufficiently stable to survive harsh environmental conditions. Importantly, they must be able to undergo rapid structural changes in response to appropriate cellular stimuli in order to accomplish crucial steps in cell entry. We are investigating the structural changes in adenoviruses that allow virions to penetrate the endosomal membrane. We found that the

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

capsids of adenovirus type 5 undergo significant conformational changes in response to mild heat treatment (45°C for 10 minutes). Specifically, the vertex region including the penton base, fiber, and protein IIIa are removed upon heating or exposure to low pH. In contrast, a temperature-sensitive mutant adenovirus that does not escape the early endosome does not undergo these structural alterations. Interestingly, compared with wild-type adenovirus type 5, this mutant also has reduced capacity to disrupt model membranes after exposure to low pH buffer. These findings suggest that specific alterations in the adenovirus capsid are required to initiate membrane association and, ultimately, endosome penetration. Further biochemical and structural studies are in progress to investigate the mechanisms of adenovirus cell entry.

## PUBLICATIONS

**Goosney, D.L., Nemerow, G.R.** Adenovirus infection: taking the back roads to viral entry. *Curr. Biol.* 13:R99, 2003.

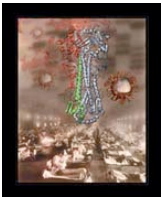
**Hsia, D.A., Mitra, S.K., Hauck, C.R., Streblow, D.N., Nelson, J.A., Ilic, D., Huang, S., Li, E., Nemerow, G.R., Leng, J., Spenser, K.S.R., Cheresch, D.A., Schlaepfer, D.D.** Differential regulation of cell motility and invasion by FAK. *J. Cell Biol.* 160:753, 2003.

**Stewart, P.S., Dermody, T., Nemerow, G.R.** Virus-receptor complexes: *In: Virus Structures*. Chiu, W., Johnson, J.E. (Eds.). Academic Press, San Diego, *in press*.

**Von Seggern, D.J., Augilar, E., Kinder, K., Fleck, S.K., Gonzelez Armas, J.C., Stevenson, S.C., Ghazal, P., Nemerow, G.R., Friedlander, M.** In vivo transduction of photoreceptors or ciliary body by intravitreal injection of pseudotyped adenoviral vectors. *Mol. Ther.* 7:27, 2003.

**Wu, E., Pache, L., Von Seggern, D.J., Mullen, T.M., Mikyas, Y., Stewart, P.L., Nemerow, G.R.** Flexibility of the adenovirus fiber is required for efficient receptor (CAR) interaction. *J. Virol.* 77:7225, 2003.





## 2004

---

### Adenovirus Penetration of Host-Cell Membranes

C.M. Wiethoff, H. Wodrich, L. Gerace, G.R. Nemerow

Adenoviruses are a useful tool for investigating fundamental molecular and cellular processes, and replication-defective adenovirus vectors are currently being used in about 28% of viral gene therapy trials in the United States. Little is known about how nonenveloped viruses, adenoviruses in particular, disrupt cell endosomes to gain access to the cytoplasm and subsequently translocate to the nucleus.

In recent studies, we discovered protein VI is a membrane lytic factor associated with the adenovirus inner capsid. Interestingly, adenovirus capsids undergo a marked conformational change in response to external stimuli that partially mimics the low pH environment of the endosome. Treatment of purified capsids with low pH buffers (e.g., pH 5.5) or with mild heat (e.g., 45°C) caused release of the vertex region composed of the fiber, penton base, protein IIIa, peripentonal hexons, and protein VI. In contrast, these same treatments did not induce disassembly of the capsids of a temperature-sensitive adenovirus mutant. Virions of the mutant virus also did not escape host-cell endosomes. Wild-type adenovirus but not the mutant virus also caused pH-dependent disruption of model membranes (liposomes).

Importantly, preheating wild-type, but not mutant, virions released a pH-independent lytic factor. Immunodepletion studies indicated that this membrane lytic factor was associated with protein VI. Further studies indicated that recombinant protein VI, but not a truncated form of the molecule lacking a predicted N-terminal amphipathic  $\alpha$ -helix, caused pH-independent membrane disruption.

Thus, our current working model of adenovirus-induced membrane disruption suggests that low pH induces disassembly of the viral capsid, allowing the release of protein VI from beneath the peripentonal hexons and resulting in membrane disruption. In further biochemical, structural, and infectivity studies, we will examine the precise mechanisms involved in adenovirus-mediated membrane disruption. This information should shed further light on interactions between adenoviruses and host cells and perhaps aid in the design of gene delivery vectors.

---

### The Role of CD46 in Adenovirus Ocular Infections

E. Wu, L. Pache, M. Martinez, R. Nepomenceno

Most of the 51 adenovirus serotypes use the coxsackievirus-adenovirus receptor to attach to host cells. However, certain types of human adenovirus associated with immunodeficiency (e.g., adenovirus type 35, subgroup B) or severe ocular infections (e.g., adenovirus type 37, subgroup D) use CD46, also known as membrane cofactor protein. CD46 is a 50- to 60-kD membrane glycoprotein that is expressed on many cell types and, interestingly, serves as a receptor for diverse bacterial and viral pathogens.

In a continuation of earlier studies, we are investigating the precise mechanisms involved in the interaction between CD46 and adenoviruses. Our current model suggests that CD46 associates as a homotrimer with the trimeric fiber knob of adenovirus type 37 in an end-to-end fashion, thereby burying a large surface area that allows high-affinity interaction with the short and rigid fiber molecule. In support of this model, we found that the top of the CD46 trimer contains 3 negatively charged glutamic acid residues within or close to the epitope recognized by an antibody that neutralizes adenovirus type 37. These residues are about 21–26 Å from their respective 3-fold symmetry axis, making them complementary with a positively charged lysine residue (K240) that we previously

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

showed regulated adenovirus type 37 binding and infection. In further molecular, structural, and biochemical studies, we will test this model to increase our understanding of interactions between adenoviruses and CD46.

In addition, we plan to investigate why CD46 has been usurped by diverse microbial pathogens. One possibility is that CD46 ligation downregulates the host immune response in favor of the pathogen. Although adenovirus type 5 vectors can induce potent host inflammatory reactions, we found that adenovirus type 5 particles pseudotyped with the adenovirus type 37 or type 35 fiber downregulated IL-12 production in human peripheral blood mononuclear cells. Studies are in progress to discover the mechanism involved in IL-12 regulation by CD46-tropic adenoviruses and to determine whether this regulation influences viral clearance from the host.

## PUBLICATIONS

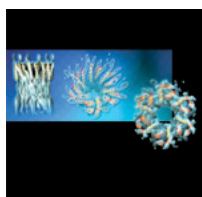
**Nicklin, S.A., Dishart, K.L., Buening, H., Reynolds, P.N., Hallek, M., Nemerow, G.R., Von Seggern, D.J., Baker, A.H.** Transductional and transcriptional targeting of cancer cells using genetically engineered viral vectors. *Cancer Lett.* 201:165, 2003.

**Stewart, P.L., Dermody, T.S., Nemerow, G.R.** Structural basis of nonenveloped virus cell entry. *Adv. Protein Chem.* 64:455, 2003.

**Trauger, S.A., Wu, E., Bark, S.J., Nemerow, G.R., Siuzdak, G.** The identification of an adenovirus receptor by using affinity capture and mass spectrometry. *Chembiochem* 5:1095, 2004.

**Wu, E., Nemerow, G.R.** Virus yoga: the role of flexibility in virus host cell recognition. *Trends Microbiol.* 12:162, 2004.

**Wu, E., Trauger, S.A., Pache, L., Mullen, T., Bark, S.B., Von Seggern, D.J., Siuzdak, G., Nemerow, G.R.** Membrane cofactor protein is a receptor for adenoviruses associated with epidemic keratoconjunctivitis. *J. Virol.* 78:3897, 2004.



## 2005

---

### Structural Analyses of an Adenoviral Vector Targeted to Hematopoietic Cells

S. Saban,\* R.R. Nepomuceno, G.R. Nemerow, P.L. Stewart\*

\* *Vanderbilt University Medical Center, Nashville, Tennessee*

Despite recent advances in the uses of adenovirus vectors for vaccines and gene delivery, we still lack basic knowledge of the structure of intact adenovirus particles. In recent studies, we used electron cryomicroscopy and image reconstruction to determine the 3-dimensional structure of an adenovirus vector, Ad35F, at 9-Å resolution. This viral vector recognizes the receptor CD46, a member of the complement regulatory protein family, thereby allowing improved gene delivery to human hematopoietic cells that express this receptor.

Important new advances in data acquisition and image processing resulted in major improvements in resolution compared with the resolution in earlier studies. For example, electron cryomicroscopy density was observed for hexon residues missing from the crystal structure that included hypervariable regions and the epitope of a neutralizing antibody. On the inner capsid surface, density was revealed at the base of the hexons and below the penton base that most likely correspond to minor adenovirus proteins, including protein VI.

On the basis of the new structural information, we proposed a new model for Ad35F. In particular, the model presents 2 possible orientations for protein IX, either binding on the capsid surface or extending away from the capsid, consistent with the use of the C terminus of protein IX for the insertion of exogenous ligands to redirect adenovirus vectors to alternative receptors. These studies increase our knowledge of adenovirus capsid assembly and antibody neutralization and thus may promote further improvements in gene delivery to hematopoietic cell types.

---

### Targeting a General Biochemical Pathway in Viral Infections Via Cyclic D,L- $\alpha$ -Peptides

W.S. Horne, C.M. Wiethoff, C. Cui, K.M. Wilcoxon, M. Amorin, M.R. Ghadiri, G.R. Nemerow

Diverse human viruses have coevolved to exploit the acidification of endosomal compartments to gain entry into host cells. Recently, we used a supramolecular approach to selectively target and inhibit viral infections through this central pathway. We used a high-throughput screen with an adenovirus vector encoding green fluorescent protein to select an 8-residue cyclic D,L- $\alpha$ -peptide from a directed combinatorial library that specifically inhibited the development of low pH inside endocytic vesicles, thereby arresting escape of virus from these compartments. The peptide had no adverse effect on cell viability and was only able to exert its inhibitory activity when added to cells in the presence of the virus. Confocal fluorescence microscopic studies with labeled adenovirus particles indicated that the peptide did not hinder viral attachment or entry but rather extinguished the pH gradient inside cell endosomes. Influenza virus that uses a mode of entry similar to that of adenovirus was also inhibited by the peptide. Our results suggest that self-assembling cyclic peptides may provide a broad-spectrum and alternative approach to the design of antiviral drugs.

## Inhibiting Expression of Proinflammatory Cytokines With Adenoviruses

M. Iacobelli-Martinez, R.R. Nepomuceno, J. Connolly,\* G.R. Nemerow

\* *Isis Pharmaceuticals, Inc., Carlsbad, California*

Most adenovirus serotypes bind to host cells via the coxsackievirus-adenovirus receptor, but subgroup B and subgroup D (adenovirus 37) viruses recognize the receptor CD46. Interestingly, diverse microbial pathogens that use CD46 for infection downregulate the expression of IL-12, a cytokine involved in both the innate and the adaptive immune responses. We determined whether adenovirus serotypes that use CD46 alter the expression of proinflammatory cytokines.

We found that subgroup B adenoviruses type 16 and 35 and subgroup D adenovirus type 37, but not subgroup C adenoviruses type 2 or 5, significantly reduced expression of IL-12 by peripheral blood mononuclear cells stimulated by IFN- $\gamma$  and lipopolysaccharide. IL-12 mRNA, as well as mRNA encoding other mediators, such as IL-1 $\alpha$ , IL- $\beta$ , the receptor for IL-1 $\alpha$ , and IL-6, were also downregulated upon interaction with adenoviruses that use CD46. Analysis of transcription factor activity required for cytokine expression indicated that adenoviruses that used CD46 preferentially inhibited the DNA-binding activity of the transcription factor CCAAT/enhancer-binding protein  $\beta$  (C/EBP- $\beta$ ). Expression of C/EBP- $\beta$  protein induced by IFN- $\gamma$  was also impaired by adenoviruses that use CD46, consistent with the reduced DNA-binding activity of C/EBP- $\beta$ . Interference with IFN- $\gamma$  signaling events by adenoviruses that use CD46, but not adenoviruses that use the coxsackievirus-adenovirus receptor, revealed a potentially critical difference in the host immune response against adenovirus vectors, a situation that has implications for gene delivery and vaccine development.

### PUBLICATIONS

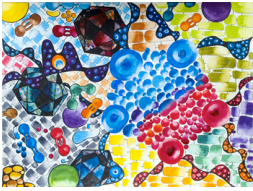
**Horne, W.S., Wiethoff, C.M., Cui, C., Wilcoxon, K.M., Amarin, M., Ghadiri, M.R., Nemerow, G.R.** Antiviral cyclic D,L- $\alpha$ -peptides: targeting a general biochemical pathway in virus infections. *Bioorg. Med. Chem.* 13:5145, 2005.

**Hsu, C., Boysen, M., Gritton, L.D., Frosst, P.D., Nemerow, G.R., Von Seggern, D.J.** In vitro dendritic cell infection by pseudotyped adenoviral vectors does not correlate with their in vivo immunogenicity. *Virology* 332:1, 2005.

**Iacobelli-Martinez, M., Nepomuceno, R.R., Connolly, J., Nemerow, G.R.** CD46-utilizing adenoviruses inhibit C/EBP $\beta$ -dependent expression of proinflammatory cytokines. *J. Virol.* 79:11259, 2005.

**Saban, S.D., Nepomuceno, R.R., Gritton, L.D., Nemerow, G.R., Stewart, P.L.** CryoEM structure at 9Å resolution of an adenovirus vector targeted to hematopoietic cells. *J. Mol. Biol.* 349:526, 2005.

**Wiethoff, C.M., Wodrich, H., Gerace, L., Nemerow, G.R.** Adenovirus protein VI mediates membrane disruption following capsid disassembly. *J. Virol.* 79:1992, 2005.



## 2006

---

### Toll-like Receptor 9 Signaling by Adenoviruses That Use CD46 Receptors

**M. Martinez-Iacobelli, G.R. Nemerow**

**H**uman adenoviruses are potent activators of the innate immune response, a feature that limits their use for in vivo gene transfer. However, the precise mechanisms by which different adenoviruses trigger innate immunity have not been fully elucidated. In recent studies, we analyzed different types of adenoviruses that use coxsackievirus-adenovirus receptors or CD46 as their primary receptors for the ability of the viruses to trigger innate immune responses as measured by production of type I interferon in human peripheral blood mononuclear cells.

We found that adenoviruses that use CD46 preferentially induced production of IFN- $\alpha$  and that this finding most likely was due to activation of Toll-like receptor 9, because an oligonucleotide antagonist of the receptor inhibited cytokine expression. Moreover, empty/immature adenovirus particles that lacked double-stranded DNA did not induce interferon production even though they were fully capable of binding to and entering host cells. In further studies, we found that epithelial cells that supported equivalent levels of infection by adenoviruses that used coxsackievirus-adenovirus receptors and by adenoviruses that used CD46 produced significantly higher amounts of interferon upon infection by the viruses that used CD46.

These findings indicate that distinct receptor-mediated entry pathways may play a pivotal role in activation of Toll-like receptor 9 by adenovirus. The findings also have implications for the development of safer adenovirus vectors for clinical applications.

---

### Improving Adenovirus Transduction of Human Myeloid Cells

**R. Nepomuceno, L. Pache, G.R. Nemerow**

**D**endritic cells are ideal targets for immunomodulatory regimens to treat genetic or acquired diseases. However the lack of coxsackievirus-adenovirus receptors on dendritic cells has stymied transfer of genes to these cell types via type 5 adenoviruses. In recent studies, we found that the fiber knob derived from adenovirus type 37 (37FK) could significantly enhance adenovirus-mediated gene transfer to primary human monocytes and to dendritic cells but not to T and B lymphocytes. Fiber knobs derived from other adenovirus strains, including type 5 and type 16, did not have this ability. Enhancement of gene transfer by 37FK depended on sialic acid, because removal of sialic acid residues by treatment with neuraminidase abrogated gene transfer. Moreover, lectins with specificity for  $\alpha$ 2,6-linked sialic acid residues, but not lectins with specificity for  $\alpha$ 2,3-linked sialic acid residues, could inhibit gene transfer by 37FK. In further investigations, we found that 37FK bound directly to adenovirus particles and thereby increased virus binding to monocyte cells.

We concluded that an electrostatic interaction between the positively charged 37FK and the negatively charged virus capsid and cell-surface sialic acid residues results in the formation of a ternary complex that potentiates adenovirus infection and gene transfer. These findings may point the way for improving gene delivery to dendritic cells.

## Adenovirus-Mediated Disruption of Endosomes and Development of Nanoparticle Delivery Methods

J.G. Smith, C. Wiethoff,\* S. Police,\*\* C.Y. Lai, V. Kickhoefer,\*\*\* L. Rome,\*\*\* G.R. Nemerow

\* *Loyola University Medical Center, Maywood, Illinois*

\*\* *Cell Genesys, Inc., San Francisco, California*

\*\*\* *University of California, Los Angeles, California*

The mechanisms by which nonenveloped viruses, including adenovirus, penetrate the barrier of the host cell endosomal membrane are not well understood. In previous studies, we identified an internal capsid protein, designated protein VI, that may mediate the disruption of the early endosome upon partial disassembly of the virion at low pH. To test this hypothesis, we recently examined the infectivity of a panel of mutant adenoviral particles that contain single amino acid substitutions in the putative membrane-reactive domain of protein VI.

Using a quantitative fluorescence imaging device, we found that several of the particles with mutant protein VI molecules had mildly reduced infectivity compared with that of wild-type virions. Further biochemical assays revealed that the reduced infectivity was not due to defects in virion assembly or disassembly. Currently, we are evaluating the membrane lytic and endosome-disrupting properties of the mutant adenoviruses; we expect that some of the mutants will have defects in these activities. In keeping with this expectation, we found that mutations in protein VI that affect virus infection also reduce binding of protein VI to liposomes. Finally, efforts are under way to determine if wild-type protein VI molecules can be incorporated into naturally occurring nanoparticles in an attempt to improve gene delivery to host cells.

---

## Structure Analyses of Adenovirus via Electron Cryomicroscopy and X-ray Diffraction

S. Saban,\* P. Stewart,\* V. Reddy, G.R. Nemerow

\* *Vanderbilt University School of Medicine, Nashville, Tennessee*

Adenovirus is one of the largest macromolecular complexes whose structure has been analyzed by using electron cryomicroscopy or x-ray diffraction. In ongoing studies, we are using electron cryomicroscopy and images of approximately 2000 particles to determine the adenovirus structure to 6–7 Å. At this level of resolution, we can clearly resolve multiple  $\alpha$ -helices present in the penton base, protein VI, hexon, and protein IIIa. These studies have also revealed the location and potential associations of the adenovirus proteins located on the inner capsid surface that help stabilize the virus before its disassembly in the early endosome.

The pseudoatomic model generated from the electron cryomicroscopy data was used to facilitate structural analyses of the virus by x-ray diffraction. We found that large single crystals of adenovirus diffracted to about 5-Å resolution at different synchrotron beam lines. Recent studies indicated that the adenovirus crystals can be frozen, thereby allowing the collection of nearly complete electron cryomicroscopy data sets from single crystals. Generation of electron density maps from x-ray diffraction data revealed distinct features of the inner core of the virus. This new information may provide further insights into the assembly and disassembly of adenovirus as well as the mode of viral DNA uncoating and recognition by Toll-like receptor 9.

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

## PUBLICATIONS

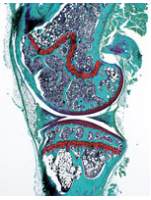
**Maginnis, M.S., Forrest, J.C., Kopecky-Bromberg, S.A., Dickeson, S.K., Santoro, S.A., Zutter, M.M., Nemerow, G.R., Bergelson, J.M., Dermody, T.S.**  $\beta_1$  Integrin mediates internalization of mammalian reovirus. *J. Virol.* 80:2760, 2006.

**Nepomucena, R., Pache, I., Nemerow, G.R.** Enhancement of gene transfer to human myeloid cells by adenovirus-fiber complexes. *Mol. Ther.*, *in press*.

**Nicklin, S.A., Wu, E., Nemerow, G.R., Baker, A.H.** The influence of adenovirus fiber structure and function on vector development for gene therapy. *Mol. Ther.* 12:384, 2005.

**Saban, S.D., Silvestry, M., Nemerow, G.R., Stewart, P.L.** Visualization of  $\alpha$ -helices in a 6 Å resolution cryoEM structure of adenovirus allows refinement of capsid protein assignments. *J. Virol.*, *in press*.

**Wodrich, H., Cassany, A., D'Angelo, M.A., Guan, T., Nemerow, G., Gerace, L.** Adenovirus core protein pVII is translocated into the nucleus by multiple import receptor pathways. *J. Virol.* 80:9608, 2006.



## 2007

---

### Unique Mechanisms of Virus Neutralization by the Adaptive and Innate Immune Systems

G.R. Nemerow, J.G. Smith, R. Ralston\*

\* *Schering-Plough Corporation, Kenilworth, New Jersey*

Control of virus infection by high-affinity neutralizing antibodies often occurs via intervention in the earliest steps in cell entry, such as receptor blockade or membrane penetration. In contrast, we found that a neutralizing monoclonal antibody directed to the major outer capsid protein (hexon) of adenovirus does not interfere with attachment of virus to cell receptors, internalization of virus, or escape of virions from endosomal compartments. Instead, the antibody blocks a relatively late step in cell entry involving transport of partially disassembled adenovirus particles to the nucleus mediated by dynein motors and microtubules. This entry step is also used by diverse viral pathogens for entry and/or cell egress. In biochemical studies, we showed that the neutralizing antibody substantially increased virus association with microtubules and microtubule-associated proteins. Further studies are in progress to pinpoint the defect in cytoplasmic transport of virus particles. Our results reveal an unanticipated mode of antibody-mediated virus neutralization.

Specific elements of the innate immune system also contribute to virus inactivation, although the mechanisms involved are not fully elucidated. We recently showed that human  $\alpha$ -defensin 5, a small, naturally occurring antimicrobial peptide, causes dose-dependent inhibition of adenovirus infection. This peptide inhibits multiple types of adenovirus, including types 5 and 35. In mechanistic studies, we showed that the defensin does not restrict virus attachment or internalization but instead efficiently blocks escape of virus from the early endosome. Human  $\alpha$ -defensin 5 stabilizes the virus particle, as indicated by resistance to temperature-mediated capsid disassembly. Increased capsid stability impedes the release of the membrane lytic protein VI, thereby blocking escape of virions from endosomes. Further studies are in progress to solve the structure of human  $\alpha$ -defensin 5 in complex with adenovirus. The knowledge gained from these studies not only will help define the role of the innate immune response in viral infections but also may lead to the development of novel antiviral compounds that restrict late events in virus entry.

---

### Biochemical and Genetic Analyses of Adenovirus-Mediated Membrane Penetration

G.R. Nemerow, J.G. Smith, C. Moyer, C. Wiethoff,\* C.-Y. Lai\*\*

\* *Loyola University Medical Center, Maywood, Illinois*

\*\* *Dupont Central Research and Development, Wilmington, Delaware*

Compared with the accumulated knowledge on membrane fusion by enveloped viruses, relatively little information is available on the mechanisms by which nonenveloped viruses penetrate cell membranes. We showed that protein VI, a capsid protein located in the interior of the adenovirus capsid, plays a key role in disruption of host cell membranes. During cell entry, the adenovirus vertex region undergoes disassembly, allowing release of protein VI from the interior of the virus concomitant with disruption of the endosome. In recent studies, we found that mutations introduced into the putative lipid-binding domain of protein VI reduce the membrane lytic activity of the protein.

Studies are in progress to introduce these mutations directly into the protein VI-coding region in adenovirus DNA to create mutant viruses with decreased infectivity and endosome-disrupting activity. In further investigations, we will use x-ray diffraction techniques to study the structure of wild-type and mutant forms of protein VI. Finally, protein VI has been incorporated into cell-derived nanoparticles (vaults) to develop novel cargo devices for gene transfer. These studies should increase our knowledge of how nonenveloped viruses penetrate cell membranes and may facilitate the development of nonviral methods of gene transfer.



## Structure Analyses of Wild-type and Mutant Adenoviruses

G.R. Nemerow, S. Saban,\* P.L. Stewart,\* V. Reddy, T. Mullen

\* *Vanderbilt University Medical Center, Nashville, Tennessee*

We recently determined the structure of a human adenoviral vector at 6.9-Å resolution, one of the largest macromolecular complexes analyzed by using electron cryomicroscopy. These studies allowed us to refine the location of multiple adenovirus capsid proteins, including protein VI, a molecule that plays a role in membrane disruption during cell entry. The results also suggested that protein IIIa acts as a linchpin for the assembly of the virus vertex region, which includes penton base, fiber, proteins IIIa and VIII, and the peripentonal hexons.

To gain a better understanding of adenovirus assembly and disassembly, we have also used electron cryomicroscopy to analyze the structure of a temperature-sensitive mutant adenovirus designated *ts1*. This virus is hyperstable and contains multiple unprocessed precursor capsid proteins. Consequently, *ts1* particles do not undergo disassembly in the endosome and lack the ability to escape this compartment during infection. In preliminary structure analyses, the *ts1* particle had a much stronger and well-defined density in the core region than the wild-type virus did. Our working hypothesis is that one or more of the unprocessed precursor capsid proteins links the core region with the outer surface of the virion, thereby resulting in a stronger reconstruction of the core. Further analyses at 10-Å resolution are planned to gain further insights into adenovirus assembly and disassembly.

To complement the electron cryomicroscopy structural studies, we have produced crystals of intact virus particles that diffract to approximately 5-Å resolution. We have also generated electron density maps of adenovirus particles at 7.5-Å resolution that reveal striking features of the virion core, including the putative location and arrangement of the viral chromosome. Further studies are in progress to improve resolution to reveal the precise location and orientation of the viral capsid proteins and the adenovirus DNA within the core.

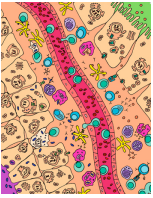
### PUBLICATIONS

**Iacobelli-Martinez, M., Nemerow, G.R.** Preferential activation of Toll-like receptor nine by CD46-utilizing adenoviruses. *J. Virol.* 81:1305, 2007.

**Nepomuceno, R., Pache, L., Nemerow, G.R.** Enhancement of gene transfer to human myeloid cells by adenovirus-fiber complexes. *Mol. Ther.* 15:571, 2007.

**Saban, S.D., Silvestry, M., Nemerow, G.R., Stewart, P.L.** Visualization of  $\alpha$ -helices in a 6-angstrom resolution cryoelectron microscopy structure of adenovirus allows refinement of capsid protein assignments. *J. Virol.* 80:12049, 2006.

**Stewart, P.L., Nemerow, G.R.** Cell integrins: common receptors for diverse viral pathogens. *Trends Microbiol.*, *in press*.



## 2008

### Molecular and Structural Basis of Adenovirus Association With CD46

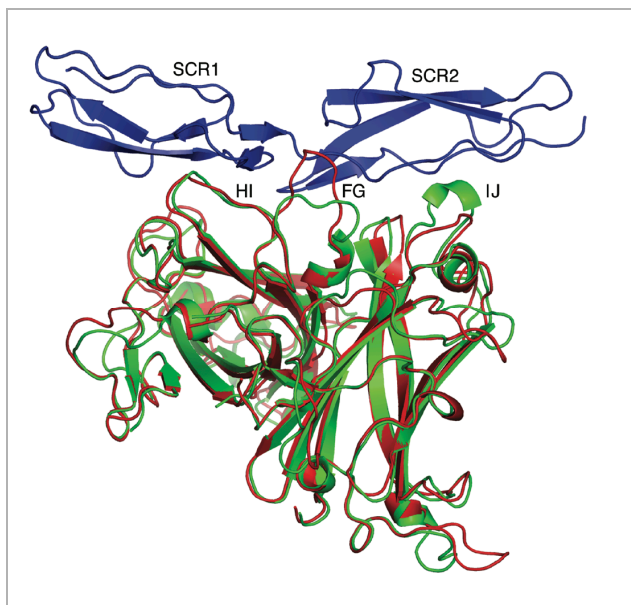
G.R. Nemerow, L. Pache, S. Venkataraman,\* V.S. Reddy\*

\* *Department of Molecular Biology, Scripps Research*

Adenovirus remains a major vector for gene transfer and vaccine delivery for the treatment or prevention of multiple human diseases. The majority of clinical applications involve the use of adenovirus type 5—based vectors; however, preexisting immunity to this serotype and the ability of type 5 adenoviruses to induce a potent proinflammatory response have hampered widespread use of type 5 vectors. Consequently, alternative adenovirus vectors based on species B viruses, which appear to avoid these problems, are now being explored. Unlike adenovirus type 5, which uses coxsackievirus-adenovirus receptor as its primary receptor, species B1 and B2 adenoviruses (e.g., adenovirus types 11, 35, and 16) use CD46, a member of the family of complement regulatory molecules. Receptor interactions are mediated by the fiber protein, which protrudes from each of the 12 vertices of the viral capsid.

Recently, we compared the molecular interactions of CD46 with 2 B2 species of adenoviruses: 11 and 35. We solved the crystal structure of the adenovirus 35 fiber knob domain at 2.7-Å resolution and constructed a model of the fiber knob in complex with the 2 short consensus repeat (SCR) domains of CD46 on the basis of a similar model previously reported for a complex consisting of adenovirus 11 and CD46. Although the structure of the adenovirus 35 fiber knob differed subtly (i.e., a shorter IJ loop) from the structure of the adenovirus 11 fiber knob, both fibers bound the receptor in a similar manner and with similar binding efficiencies, as indicated by functional and biochemical analyses. Thus, members of species B2 adenovirus appear to have highly conserved receptor-binding fibers.

In contrast, we discovered that the fiber protein of adenovirus 16, a member of the B1 species, has a markedly reduced affinity for CD46, although virions with the adenovirus 16 fiber can still use CD46 on host cells, albeit with reduced capacity. We determined the crystal structure of the adenovirus 16 fiber knob and found that it has several structural features distinct from those of adenovirus 11 or adenovirus 35 (Fig. 1). In particular, adenovirus 16 fiber knob has an outer loop, designated FG, that is 2 amino acids longer than the corresponding loops in other B2 adenovirus fibers. Further mutagenesis studies and functional analyses revealed that the longer FG loop of adenovirus 16 likely interferes with CD46 association via steric hindrance. Other types of B1 adenovirus also have this structural feature, a finding that may explain the lower efficiency of CD46 binding by certain adenovirus types. Our recent studies contribute new knowledge on the molecular basis of CD46 use by various types of species B adenovirus and may point the way to optimizing receptor interactions on target cells with alternative adenovirus vectors.



**Fig. 1.** Ribbon diagram of 2 subunits of the adenovirus 16 fiber knob (red) superposed on the complex consisting of adenovirus 11 fiber knob (green) and SCR1 and SCR2 of CD46 (blue). FG, HI, and IJ loops of adenovirus 16 are indicated.

# Nemerow Laboratory Scientific Reports 1997-2008

Excerpts from TSRI Scientific Report - Copyright © [TSRI](#)

---

## Generation of Vault Nanoparticles With Enhanced Cell Delivery Capacity

G.R. Nemerow, C.-Y. Lai,\* T. Mullen, C. Wiethoff,\*\* V.A. Kickhoefer,\*\*\* L.H. Rome\*\*\*

\* *Dupont Central Research and Development, Wilmington, Delaware*

\*\* *Loyola University Medical Center, Maywood, Illinois*

\*\*\* *University of California, Los Angeles, California*

Although use of viral vectors in gene transfer has been successful in some trials, the host immune response and problems with toxicity have limited the use of these agents. In collaboration with L.H. Rome, University of California, Los Angeles, we have inserted the membrane lytic domain of adenovirus protein VI inside naturally occurring cellular nanoparticles known as vaults to increase the ability of the particles to penetrate membranes. We found that the membrane lytic activity of protein VI was retained when the protein was incorporated into vault particles and that these recombinant vesicles could enhance the delivery of a toxin or a cDNA plasmid into murine macrophages in vitro.

These findings set the stage for further exploration of recombinant vault particles as gene/drug delivery devices with reduced toxicity and immunogenicity. The findings should also allow us to more fully explore the membrane-disrupting activity of protein VI.

---

## Blockade of Adenovirus Cell Entry and Infection by Human $\alpha$ -Defensins

G.R. Nemerow, J.G. Smith

Naturally occurring antimicrobial peptides, including the  $\alpha$ -defensins such as human  $\alpha$ -defensin 5 (HD5), make up part of the innate immune response that serves as a first line of protection against invading bacterial pathogens. Defensins are often secreted from mucosal surfaces via specific cell types, such as the Paneth cells of the small intestine, in response to infection. These peptides can achieve a high local concentration that is sufficient to perturb the lipid envelope of bacterial agents. Interestingly, recent reports indicate that defensins can neutralize a variety of human viruses, including ones that lack an outer envelope, although the mechanism involved in this process has not been characterized.

We found that human defensins HD5 and HNP-1 cause dose-dependent inactivation of adenovirus. These peptides do not block virus binding to cells or prevent internalization of virus. Instead HD5 causes accumulation of adenovirus particles in early and late endosomes, and this accumulation is associated with the failure of virions to undergo partial disassembly and release of the membrane lytic protein VI molecule. This novel mode of virus neutralization may extend to other nonenveloped viruses, including human papillomavirus, that accumulate in endosomes in the presence of HD5. Further studies are under way to examine the molecular and structural features of defensin binding to adenovirus and the immune responses to adenoviruses complexed to defensins.

### PUBLICATIONS

**Pache, L., Venkataraman, S., Nemerow, G.R., Reddy, V.S.** Conservation of fiber structure and CD46 usage by subgroup B2 adenoviruses. *Virology* 375:573, 2008.

**Pache, L., Venkataraman, S., Reddy, V.S., Nemerow, G.R.** Structural variations in species B adenovirus fibers impact CD46 association. *J. Virol.* 82:7923, 2008.

**Smith, J.G., Nemerow, G.R.** Mechanism of adenovirus neutralization by human  $\alpha$ -defensins. *Cell Host Microbe* 3:11, 2008.

**Stewart, P.L., Nemerow, G.R.** Cell integrins: commonly used receptors of diverse viral pathogens. *Trends Microbiol.* 15:500, 2007.