Fourth International Conference on Foamy Viruses

Organized by the Centers for Disease Control and Prevention (CDC)

March 14 - 16, 2002
D. Abbott Turner Center
1703 Clifton Road
Atlanta, Georgia 30329, USA

Program and Abstracts Book

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Thursday, March 14

6:00-7:00 p.m.

Registration

7:00 p.m.

Welcoming Remarks

Walid Heneine, Ph.D.
Thomas Folks, Ph.D.
HIV and Retrovirology Branch
Division of AIDS, STD, and TB Laboratory Research
National Center for Infectious Diseases
Centers for Disease Control and Prevention

Opening Address

Brian WJ Mahy, M.S., Ph.D., Sc.D., D.Sc.
Senior Scientific Research Advisor
National Center for Infectious Diseases
Centers for Disease Control and Prevention

Friday, March 15

Session 1: ***Foamy Virus-Host Interaction***

Chairs: Dieter Neumann-Haefelin and Walid Heneine

8:30-10:00 a.m.

1.1 Increased Rate of Simian Foamy Virus Infection among Persons Occupationally Exposed to Nonhuman Primates

William M. Switzer,[1] Shanmugam Vedapuri,[1] Paul Sandstrom,[1] Vinoid Bhullar,[1] JoAnn Yee,[2] Nicholas Lerche,[2] Roumiana Boneva,[1] Louisa Chapman,[1] Thomas Folks,[1] and Walid Heneine.[1]
[1]Centers for Disease Control and Prevention, Atlanta, Georgia; [2]University of California, Davis, California, USA.

1.2 Human Infection with Simian Foamy Viruses: Preliminary Results from a Long-term Follow-up Study

R.S. Boneva, W.M. Switzer, T. Spira, V. Shanmugam, V. Bhullar, J.E. Cummins Jr, A.I. Hussain, W. Heneine, T.M. Folks, and L.E. Chapman.
Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

1.3 Evidence of Zoonotic Transmission of SFV from Macaques

James I. Brooks,[1] Erling W. Rud,[1] Jonathan Smith,[1] and Paul A. Sandstrom.[1,2]
[1]Health Canada, Ottawa, Canada; [2]Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

1.4 Virus Recovery from the Oral Mucosa and Evidence of Viral Quasispecies and Tissue Compartmentalization in an SFVcpz-infected Person
W.M. Switzer, V. Shanmugam, V. Bhullar, T.M. Folks, R.S. Boneva, L.E. Chapman, and W. Heneine.
Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

1.5 Cell-Mediated Immune Responses in Humans Infected with Simian Foamy Viruses
Steffanie Sabbaj,[1] G. Douglas Ritter,[1] James Cummins,[2] Roumiana Boneva,[2] Louisa Chapman,[2] and Paul Goepfert.[1]
[1]University of Alabama at Birmingham, Birmingham, Alabama; [2]Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

1.6 Humoral Immune Responses in the Oral Mucosa of Humans Occupationally Infected with SFVcpz
James E. Cummins, Jr., William M. Switzer, Roumiana S. Boneva, Vedapuri Shanmugam, Vinod Bhullar, Walid Heneine, Louisa E. Chapman, Paul Sandstrom, and Charlene S. Dezzutti.
Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

10:00-10:30 a.m.
BREAK

10:30-11:45 a.m.

1.7 Molecular Characterization of the Full Length Genome of a Zoonotic Simian Foamy Virus
M.E. Callahan, K.N. Lee, P.A. Sandstrom, W.M. Switzer, W. Heneine, T.M. Folks, and S. Subbarao.
Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

1.8 Investigation of Simian Foamy Virus Transmissibility by Transfusion
R. S. Boneva,[1] A. Grindon,[2] S. Orton,[3] W. M. Switzer,[1] V. Shanmmugam,[1] V. Bhullar,[1] A. I. Hussain,[1] W. Heneine,[1] T. Folks,[1] L. E. Chapman.[1]
[1]Centers for Disease Control and Prevention, Atlanta, Georgia; [2]American Red Cross Blood Services, Southern Region, Atlanta, Georgia; [3]American Red Cross, Rockville, Maryland, USA.

1.9 Diagnosis of SFV Infection in Nonhuman Primates and Occupationally Exposed Humans by Using a Combined Antigen WB Assay (CA-WB)
A.I. Hussain,[1] V. Shanmugam,[1] V.B. Bhullar,[1] P. Sandstrom,[1,2] B. Beer,[3] D. Vallet,[4] A. Gautier-Hion,[4] Z. Tooze,[5] W. Heneine,[1] and W.M. Switzer.[1]
[1]Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [2]Health Canada, Ottawa, Canada; [3]National Institutes of Health, Rockville, Maryland, USA; [4]Ecologie Station Biologique, Paimpont, France; [5]Cercopan, Calabar, CRS, Nigeria.

1.10 Experimental Infection of Tamarins and Marmosets with Foamy Virus
E.J. Verschoor, S. Langenhuijzen, H. Niphuis, H. Weiler, and J.L. Heeney.
Biomedical Primate Research Center, Rijswijk, The Netherlands.

1.11 Infectivity Studies of Naturally Occurring Simian Foamy Viruses Isolated from Pig-tailed Macaques (*M. nemestrina*) and Rhesus Macaques (*M. mulatta*)
Arifa S. Khan, Teresa A. Galvin, Patricia Zerfas, and Theodore Bryan.
U.S. Food and Drug Administration, Bethesda, Maryland, USA.

12:00-1:30 p.m.
LUNCH

Session 2: Virology-Replication
Chairs: Maxine Linial and Matin Löchelt

1:30-3:00 p.m.

2.1 Analysis of the Feline Foamy Virus (FFV) Replication Strategy
J. Roy,[1] W. Rudolph,[1] S. Kanzler,[1] M. Heinkelein,[1,2] M. Picard-Maureau,[2] D. Lindemann,[2] O. Herchenröder,[1] and A. Rethwilm.[1]
[1]Technische Universität Dresden; [2]Universität Würzburg, Germany.

2.2 Specific Interaction of a Novel Foamy Virus Env Leader Protein with the N-Terminal Gag Domain
Thomas Wilk,[2] Verena Geiselhart,[1] Stephen D. Fuller,[2] Rolf M. Flügel,[1] and Martin Löchelt.[1]
[1]Deutsches Krebsforschungszentrum, Heidelberg, Germany; [2]University of Oxford, Oxford, United Kingdom.

2.3 Domains of Foamy Virus Gag Involved in Particle Assembly and Morphogenesis
Scott W. Eastman, and Maxine L. Linial.
Fred Hutchinson Cancer Research Center and University of Washington, Seattle, Washington, USA.

2.4 Complex Regulation of the Two Foamy Virus Promoters in Lytic and Persistent Infection
Christopher D. Meiering and Maxine L. Linial.
Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

2.5 The Highly Conserved Sequence 'TGTG' at the 5'end of U3 in Foamy Virus Genomes
T. Juretzek and A. Rethwilm.
Technische Universität Dresden, Germany.

2.6 Centrosomal Targeting of HFV Requires the Interaction of the Structural Gag Protein with LC8, the Cytoplasmic Light Chain of Dynein
C. Petit,[1] M.L. Giron,[1] P. J. Tobaly-Tapiero,[1] Bittoun,[1] Y. Jacob,[2] E. Real,[2] N. Tordo,[2] and A. Saïb.[1]
[1]Hôpital Saint-Louis and [2]Institut Pasteur, Paris, France.

3:00-3:30 p.m.
BREAK

3:30-5:15 p.m.

2.7 Identifying Bel1-Activated Human Genes and Transcriptional Regulation of Human cdkn1c Gene by the Spumaretroviral Bel 1
Rolf M. Flügel, Martin Löchelt, and Kenji Kido.
Deutsches Krebsforschungszentrum, Heidelberg, Germany.

2.8 Transcriptional Regulation of the Human p57Kip2 Gene by Sp1 and the Spumaretroviral *Trans*-activator
Kenji Kido, Martin Löchelt, and Rolf M. Flügel.
Deutsches Krebsforschungszentrum, Heidelberg, Germany.

2.9 Inter- and Intracellular Movements of the Accessory Foamy Virus Bet Protein
C.H. Lecellier,[1] M.L. Giron,[1] F. Bachelerie,[2] and A. Saïb.[1]
[1]Hôpital St Louis; and [2]Institut Pasteur, Paris, France.

2.10 Pregenomic RNA is Required to Incorporate Pol into Foamy Virus Capsids
Martin Heinkelein,[1,2] Matthias Rammling,[1] Katrin Peters,[1,2] and Axel Rethwilm[1,2]
[1]Universität Würzburg; and [2] Technische Universität Dresden, Germany.

2.11 Decrease in Processivity of a Reverse Transcriptase Catalytic-site Mutant Blocks Foamy Virus Replication
Carolyn S. Rinke,[1] Paul L. Boyer,[2] Stephen H. Hughes,[2] and Maxine L. Linial.[1]
[1]Fred Hutchinson Cancer Research Center and Department of Microbiology, University of Washington, Seattle, Washington; [2]NCI-Frederick Cancer Research and Development Center, Frederick, Maryland, USA.

2.12 Characterization of FV Isolates after AZT-resistance Induction
Marc Cartellieri,[1] Sylvia Kanzler,[1] Jacqueline Roy,[1] Wolfram Rudolph,[1] Ayalew Mergia,[2] Axel Rethwilm,[1] and Ottmar Herchenröder.[1]
[1]Technische Universitaet, Dresden, Germany; [2]University of Florida, Gainesville, FL, USA.

2.13 Foamy Virus Infectivity is Inhibited by Lysosomotropic Agents
Marcus Picard-Maureau,[1] Axel Rethwilm,[2] and Dirk Lindemann.[1]
[1]Universität Würzburg; [2]Technische Universität Dresden, Germany.

End of First Day

Saturday, March 16

Session 3: ***Foamy Virus Vectors***
Chairs: Axel Rethwilm and Thomas Folks

8:30-10:00 a.m.

3.1 Transduction of SCID Repopulating Cells by a Human Foamy Virus Vector
Neil C. Josephson,[1] George Vassilopoulos,[1] Grant D. Trobridge,[1] Greg V. Priestley,[1] Thalia Papayannopoulou,[1] Brent L. Wood,[2] and David W. Russell.[1]
[1]Department of Medicine and [2]Department of Laboratory Medicine, University of Washington, Seattle, Washington, USA.

3.2 Transduction of Hematopoietic Stem Cells (HSCs) with Human Foamy Virus (HFV) Vectors
G.S. Patton,[1] K. Parsley,[2] A.J. Thrasher,[2] and M.O. McClure.[1]
[1]Imperial College School of Medicine and [2]University College London, United Kingdom.

3.3 Efficient Transduction of Human CD34+ Cord Blood Cells with a Primate Foamy Virus Vector
C. Leurs,[1] M. Heinkelein,[2,3] D. Lindemann,[2] G. Jarmy,[2] A. Rethwilm,[3] and H. Hanenberg.[1]
[1]University of Düsseldorf; [2]University of Würzburg; [3]Technical University of Dresden, Germany.

3.4 Ex vivo Transduction by Foamy Virus-derived Vectors and Hepatic Reimplantation of Primate Hepatocytes
V. Falcone,[1] M. Heinkelein,[4] C. Dühnen,[1] P. Pisarski,[2] J. Haberstroh,[2] P. Greiner,[3] and D. Neumann-Haefelin.[1]
Departments of [1]Virology, [2]Surgery, and [3]Pediatrics, University of Freiburg, Germany; [4]University of Würzburg, Germany.

3.5 Transduction Properties of Tas-Independent Foamy Vectors and Development of Improved Vectors with Minimal *Cis*-acting Regions
Grant Trobridge, George Vassilopoulos, Jaclynn Mac, and David W. Russell.
University of Washington, Seattle, Washington, USA.

9:45-10:30 a.m.
BREAK

10:30-11:45

3.6 Foamy Viruses as Live Vaccine Vectors for HIV
T. Folks,[1] Y. Xie,[2] F.S. Hsu,[2] D.S. An,[2] K. Grovit-Ferbas,[2] and I. Chen.[2]
[1]Centers for Disease Control and Prevention, Atlanta, Georgia; and [2]University of California at Los Angeles, Los Angeles, California, USA.

3.7 Efficient Pseudotyping of Human Immunodeficiency Virus and Murine Leukemia Virus Particles with Foamy Virus Envelope Proteins
Gergely Jármy,[1] Angelika Berg,[1] Martin Heinkelein,[2] Marcus Picard-Maureau,[1] Axel Rethwilm,[2] and Dirk Lindemann.[1]
[1]Universität Würzburg; [2]Technische Universität Dresden, Germany.

3.8 Circular DNA with Two Tandem LTRs Does Not Serve as a Target for Integration by the HFV Integrase
Rebecca A. Russell,[1] Rebecca Critchley,[2] Georges Vassaux,[2] and Myra O. McClure.[1]
[1]Jefferiss Trust Research Laboratories, Wright-Fleming Institute, Imperial College School of Medicine, London, UK; and [2]Molecular Therapy Laboratory, ICRF Molecular Oncology Unit, Imperial College School of Medicine, London, UK.

3.9 The *bet* Gene of Feline Foamy Virus Is Required for Virus Replication: Implications for FFV Vector Construction
Astrid Schwantes, Alexandra Alke, Kenji Kido, Rolf M. Flügel, and Martin Löchelt.
Deutsches Krebsforschungszentrum, Heidelberg, Germany.

3.10 Analysis of Foamy Virus Gag and Pol Expression Plasmids for Packaging of Foamy Virus Gene Transfer Vectors
Martin Heinkelein,[1,2] Gregor Jarmy,[1] Matthias Rammling,[1] Jana Thurow,[1] Dirk Lindemann,[1] and Axel Rethwilm.[2]
[1]Universität Würzburg; [2]Technische Universität Dresden, Germany.

12:15 p.m.
Concluding Remarks

End of Conference

Session 1: Foamy Virus-Host Interaction

1.1 Simian Retrovirus Infections in Persons Occupationally Exposed to Nonhuman Primates

William M. Switzer,[1] Shanmugam Vedapuri,[1] Paul Sandstrom,[1] Vinoid Bhullar,[1] JoAnn Yee,[2] Nicholas Lerche,[2] Roumiana Boneva,[1] Louisa Chapman,[1] Thomas Folks,[1] and Walid Heneine.[1]

[1]Centers for Disease Control and Prevention, Atlanta, Georgia, USA; and [2]University of California, Davis, California.

Current evidence suggests that HIV-1 and HIV-2 have originated from cross species infections of simian immunodeficiency virus (SIV) from nonhuman primates (NHP). Humans remain at risk of zoonotic infections with SIV or other simian retroviruses endemic among NHP. To better assess these risks we studied persons occupationally exposed to NHP for evidence of infection with SIV, simian T cell lymphotropic viruses (STLV), simian foamy viruses (SFV), and simian type D retroviruses (SRV). 279 participants from 12 North American institutions were enrolled in a voluntary study. Serum was initially screened using Western blot (WB) assays. Peripheral blood mononuclear cells (PBMC) from reactive samples were further tested by PCR, sequence analysis, and virus isolation. 12 (4.3%) persons were WB positive for SFV and 2 (0.7%) were WB positive for SRV. One person was seropositive to both SRV and SFV. One sample was seropositive for HIV-2/SIV and was from a person with a known SIV infection. No STLV infection was identified. SFV DNA was identified by PCR in PBMC samples available from 11 SFV-seropositive persons. Phylogenetic analysis of the integrase sequence indicated SFV infections originated from an African green monkey (n=1), baboons (n=4), or chimpanzees (n=6). SFV seropositivity of a minimum of 6-24 years (mean 17.4 years) was documented for 10 persons for whom archived samples were available. All 12 SFV-infected persons were males; none appear to have disease that might be attributable to the SFV infection. Wives of six SFV-infected men were tested and remain uninfected. Attempts to identify SRV DNA by PCR and to isolate virus were unsuccessful in both SRV-positive persons, and seroreversion was subsequently documented in one person. This study documents simian retrovirus infection of persons occupationally exposed to NHP. These workers appear to be at a higher risk for infection with SFV than with SRV, SIV, or STLV. Study of this unique population may clarify the pathogenic potential and secondary transmissibility of simian retrovirus infections of humans, and may provide information facilitating worker protection.

1.2 Human Infection with Simian Foamy Viruses: Preliminary Results from a Long-term Follow-up Study

R.S. Boneva, W.M. Switzer, T. Spira, V. Shanmugam, V. Bhullar, J.E. Cummins Jr, A.I. Hussain, W. Heneine, T.M. Folks, and L.E. Chapman.
Centers for Disease Control and Prevention, Atlanta, GA.

Background: Simian foamy virus (SFV) infection has been identified in 10 of 11 seropositive men occupationally exposed to nonhuman primates (NHPs). We have begun a cohort study to characterize SFV infection and investigate the possibility of secondary transmission among humans. **Methods**: SFV-infected humans and their close contacts are eligible to enroll. At enrollment, seroreactivity and PCR positivity of SFV-infected participants are reconfirmed. Participants are questioned about demographics, occupational exposures, work practices, health status, and opportunities for transmission. Blood and body fluids (saliva, throat swabs, urine, and semen) are collected annually for clinical, virological, and immunologic testing. Enrolled contacts are only tested for SFV infection by WB and PCR. **Results**: Five of 11 eligible persons have been enrolled; 1 refused participation, and 1 was lost to follow up. The mean age at enrollment was 51 years (range 41 to 62 years). Occupations of enrollees were veterinarian (participant A), supervisor of animal care workers (B and C), and animal care worker (D and E). Reported exposure to NHPs ranged from 4 to 41 years (mean 21.2±11.2 years). All 5 reported a history of both mucocutaneous exposures to NHP body fluids and occupational injuries with skin penetration. Samples from participants A-D have been tested. SFV was successfully cultured from peripheral blood mononuclear cells from participants A and B and from a throat swab from participant A but could not be cultured from other body fluids or from other participants. Evidence of viral DNA in several body compartments is presented separately. Complete blood counts, blood chemistry, and liver function tests were within normal limits, with the following exceptions: participant A had low number of eosinophils; participant D had mildly elevated hemoglobin, hematocrit, and red cells, mild thrombocytopenia, and a CD8+ cell count just below the lower normal limit; participant C had a mildly elevated ALT. The wives of 3 participants were tested and were found to be seronegative after a median documented duration of exposure of 19 years (range 12 to 20 years). **Conclusions**: Interim analysis found no evidence of pathogenicity attributed to SFV infection and no evidence of person-to-person transmission.

1.3 Evidence of Zoonotic Transmission of SFV from Macaques

James I. Brooks,[1] Erling W. Rud,[1] Jonathan Smith,[1] and Paul A. Sandstrom.[1,2]
[1]Health Canada, Ottawa, Canada; [2]HIV/AIDS/Retrovirology Branch, CDC, Atlanta, USA

Background: Published reports have described zoonotic transmission of simian foamy virus (SFV) to humans from African green monkeys, baboons, and chimpanzees. However, previous studies have failed to detect transmission of macaque foamy virus to humans despite the popularity of macaques as experimental models. Cynomolgus macaques (*Macaca fascicularis*) represent 60% of the non-human primates used in experimental protocols in Canada and 86% of the non-human primates imported into the United States in the year 2000. **Method:** In order to screen for macaque SFV infections in humans, we carried out an anonymous, unlinked study among employees at the Health Canada primate colony extensively exposed to cynomolgus macaques, using an assay optimized to detect macaque SFV infections. After providing informed consent, Health Canada primate colony workers completed a questionnaire and provided blood samples. An immunoblot assay was developed using standardized amounts of antigen from SFV1, SFV3, SFV6, and from the cynomolgus foamy virus strain circulating in the Health Canada primate colony. Serum samples were tested in duplicate using a 1:100 dilution. Positive human serum samples were tested against individual foamy virus lysates to determine if reactivity was specific to a particular SFV strain. **Results:** 46 individuals participated in the study. 91% of the participants worked directly with cynomolgus macaques; of these, 90% had been exposed to primate body fluids. Two individuals had serological reactivity on the immunoblot assay (4.3%). Both individuals had multiple exposures, including bites, from cynomolgus and rhesus macaques. Serum from both infected humans reacted most strongly with antigen from cynomolgus and rhesus macaque foamy virus infected cells, consistent with infections of macaque origin. Molecular characterization of the provirus from isolated PBMCs is ongoing. **Conclusions:** We report evidence of zoonotic transmission of SFV from macaques. The detection of these macaque infections was likely enhanced using target antigen from the circulating strain of SFV in the Health Canada primate colony. A linked study is underway to identify infected individuals, facilitate counseling, and to pursue potential changes in the virus required for zoonotic infection.

1.4 Virus Recovery from the Oral Mucosa and Evidence of Viral Quasispecies and Tissue Compartmentalization in an SFVcpz-infected Person

W.M. Switzer, V. Shanmugam, V. Bhullar, T.M. Folks, R.S. Boneva, L.E. Chapman, and W. Heneine.
Centers for Disease Control and Prevention, Atlanta, GA USA

Surveillance for simian retroviral infection among persons occupationally exposed to nonhuman primates has confirmed simian foamy virus (SFV) infection in 10/11 seropositive workers. To date, no known pathogenicity or human-to-human transmission of SFV has been described. To better understand the potential for secondary transmission, we investigated the presence of SFV in body fluids from an SFVcpz-infected worker (Case 6). This person has a documented seropositivity of 20 yrs and was a frequent blood donor; his wife of 35 yrs is SFV-negative. We performed PCR analysis of the highly variable SFV ORF-2 region on samples from peripheral blood lymphocytes (PBLs), saliva, throat swab, and urine collected 10 months apart and on a single semen sample collected from the first time point. In addition, cells from saliva and throat and PBLs from both time points were each co-cultured with canine thymocytes. SFVcpz was isolated from both the PBLs and throat from the second time point. DNA from the cell pellets of all body fluids was PCR positive, demonstrating the presence of SFV-infected cells in all sites. Sequence analysis of multiple clones from each site revealed the presence of viral quasispecies, with intra- and inter-site divergence of $\leq$7% and $\leq$11%, respectively, with the greatest divergence observed in the PBLs. Phylogenetic analysis by the neighbor-joining method showed a clustering of the majority of the throat, saliva, and PBL quasispecies in one branch, while clones from the semen sample formed a separate, strongly supported branch suggestive of tissue-specific compartmentalization. The presence of sequence heterogeneity in Case 6 indicates a history of active viral replication and may explain the persistence of this infection. The existence of SFV-infected cells in semen and other body fluids and the recovery of infectious virus from the throat and PBLs suggest that factors other than the absence of SFV in body fluids may contribute to the observed lack of secondary transmission.

1.5 Cell-Mediated Immune Responses in Humans Infected with Simian Foamy Viruses

Steffanie Sabbaj,[1] G. Douglas Ritter,[1] James Cummins,[2] Roumiana Boneva,[2] Louisa Chapman,[2] and Paul Goepfert.[1]

[1]University of Alabama at Birmingham, Birmingham, Alabama, USA, Department of Medicine; [2]Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Humans can be infected with foamy viruses (FV) as a result of zoonotic transmission from simian hosts. While only a handful of these transmissions are documented, it appears that the infections do not produce disease, and there is no evidence of horizontal transmission of FV among humans through close contacts. One possible explanation for these observations is that FV elicits a robust cellular immune response in humans resulting in efficient control of infection, thereby preventing subsequent transmission and disease manifestations. FV-specific cell-mediated immune (CMI) responses in four individuals infected with simian foamy viruses from chimpanzees (SFV_{cpz}), in addition to two uninfected controls, were measured by stimulating fresh peripheral blood mononuclear cells (PBMC) with infectious SFV_{cpz}, recombinant vaccinia viruses expressing the SFV_{cpz} Gag and Env proteins, a recombinant vaccinia virus expressing β-galactosidase, or media alone. The number of cells producing interferon-γ (IFN-γ) was then evaluated using the ELISPOT assay. Two of the four SFV_{cpz}-infected persons had measurable responses to FV-specific proteins when cellular IFN-γ was evaluated 5 days after PBMC were stimulated with either SFV_{cpz} specific technique. PBMC derived from subjects LTF 001 and LTF 004 and stimulated with infectious SFV_{cpz} yielded 70 and 115 spot forming cells (SFC)/ 10^6 PBMC, respectively. Neither of the two uninfected controls had FV specific responses. Therefore, CMI responses, while present, were quantitatively weak. These preliminary data suggest that a robust CMI response is probably not responsible for the benign nature of FV infection as seen in humans. Further work is needed to more fully characterize the interactions of the various aspects of the immune system in response to and containment of FV infections in humans. These and future immune studies may have implications for the development of FV as vectors for gene therapy.

1.6 Humoral Immune Responses in the Oral Mucosa of Humans Occupationally Infected with SFV_{cpz}

James E. Cummins, Jr., William M. Switzer, Roumiana S. Boneva, Vedapuri Shanmugam, Vinod Bhullar, Walid Heneine, Louisa E. Chapman, Paul Sandstrom, and Charlene S. Dezzutti.
Centers for Disease Control and Prevention, Atlanta, GA (USA)

Simian foamy virus (SFV) causes persistent and apparently benign infections in naturally infected nonhuman primates. Since no known cases of human-to-human transmission or etiologically associated disease have been observed, the significance of SFV infections identified in occupationally exposed humans is unknown. Elucidation of SFV-specific mucosal immunity will increase our understanding of the potential for SFV-associated pathogenesis or human transmission. Serum (n=3) and saliva (n=2) samples were obtained from three humans infected with chimpanzee type SFV (SFV_{cpz}); serum (n=1) and saliva (n=2) samples were also obtained from three naturally SFV-infected chimpanzees. PCR detected SFV_{cpz} DNA in cells obtained from throat swabs and saliva from one human; virus was also isolated from cultured throat cells, thus indicating the presence of virus in the oral mucosa. Western blot analysis demonstrated SFV_{cpz}-specific IgA and IgG in plasma and saliva from infected humans. Preliminary results indicated strong plasma IgG reactivity (titer >>1:320) and weaker plasma IgA reactivity (titer $\leq$1:40). Although SFV_{cpz}-specific antibodies were present in human saliva, the levels were lower than those in the plasma (IgG titer $\leq$1:16, IgA titer $\leq$1:8). In chimpanzee plasma, similar anti-SFV_{cpz} IgG (IgG titer >>1:320) and higher IgA (titer $\leq$1:160) levels were obtained when compared to those in human plasma. Chimpanzee saliva had a >2-fold higher anti-SFV_{cpz} IgG titer (>1:32) and similar IgA titer ($\leq$1:16) as compared to human saliva. These results indicate reduced mucosal humoral immunity in SFV-infected humans in comparison to infected chimpanzees. Further study of human and chimpanzee SFV-specific host immunity as well as localization of virus within the oral cavity is necessary to determine the contribution, if any, to the absence of identifiable secondary transmission among humans.

1.7 Molecular Characterization of the Full Length Genome of a Zoonotic Simian Foamy Virus

M. E. Callahan, K.N. Lee, P.A. Sandstrom, W.M. Switzer, W. Heneine, T.M. Folks, and S. Subbarao.
HIV and Retrovirology Branch, Centers for Disease Control and Prevention, Atlanta GA, USA.

Surveillance programs to examine human infection with simian retroviruses have identified a significant prevalence of simian foamy virus (SFV) infections in occupationally exposed individuals. To examine the genomic stability and the adaptive changes that may occur after zoonotic transmission of SFV, we have sequenced the full-length genome of SFVhu1, originally isolated from a human infected with SFV-3, a virus of African green monkey (AGM) origin. SFVhu1 shares 87% nucleic acid identity with SFV-3 over the 13 kb genome, with *pol* and *gag* being the most conserved regions, sharing 94% and 92% amino acid identity with SFV-3, respectively. Interestingly, *env* of SFVhu1 has only 85% amino acid identity with SFV-3. The functional domains of *env*, including the signal peptide, proteolytic cleavage site, membrane spanning domain and the ER retrieval site in the transmembrane (TM) domain are conserved, sharing 95% amino acid identity in the TM domain. Phylogenetic analysis of the more variable surface domain of *env* indicates SFVhu1 is in Group 2 of AGM foamy viruses. These observations support and extend previous findings that the SFV genome is stable in zoonosis and persistent infection. Based on this sequence information, full-length molecular clones of SFVhu1 have been constructed. The frameshift mutations in *orf-2* of SFVhu1 are present in the clones. In addition, the full-length clones display *env/bet* deletions similar to those reported in human foamy virus (HFV). These SFVhu1 clones with *env/bet* deletions are infectious when Tas is expressed in *trans*.

1.8 Investigation of Simian Foamy Virus Transmissibility by Transfusion

R. S. Boneva,[1] A. Grindon,[2] S. Orton,[3] W. M. Switzer,[1] V. Shanmmugam,[1] V. Bhullar,[1] A. I. Hussain,[1] W. Heneine,[1] T. Folks,[1] L. E. Chapman.[1]

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Background: CDC surveillance identified simian foamy virus (SFV) infection in 10 of 11 SFV-seropositive male workers occupationally exposed to nonhuman primates (NHPs). Four infected workers donated blood after their retrospectively documented date of infection. We investigated whether SFV, a highly leukocyte-associated retrovirus, can be transmitted through transfusion of blood products from infected donors. **Methods:** We identified and tested recipients of blood products from one SFV-infected donor and plasma derivatives containing plasma donated by him. Testing for SFV included serology and polymerase chain reaction for integrase sequences. **Results:** Recovered plasma from 2 of 6 donations made between 1992 and 1997 was sent for manufacturing into plasma derivatives. Samples of one lot of albumin and three lots of plasma protein fraction tested SFV-negative. One of 9 identified transfusable blood components was discarded. Final disposition of one unit of leukoreduced red cells (PRF) remains undetermined. One recipient of platelets could not be tested. One recipient of packed red cells (PRC) and one recipient of fresh frozen plasma had died; however, their diagnoses did not implicate retroviral infection. Two recipients of PRC, one recipient of PRF, and one recipient of platelets tested SFV-negative 19 months to 7 years after transfusion. The transfusions took place 3 to 35 days after donation. **Conclusions**: These results do not indicate SFV transmission among humans through transfusion of blood components or plasma derivatives. However, the probability of transmission in this specific setting was low. Although there was no evidence of transmission from one SFV-infected donor, additional donor-recipient pairs will need to be studied before drawing conclusions about the potential for transfusion-associated SFV infection.

1.9 Diagnosis of SFV Infection in Nonhuman Primates and Occupationally Exposed Humans by Using a Combined Antigen WB Assay (CA-WB)

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Simian foamy viruses (SFV) belong to a genetically and antigenically related class of retroviruses that infect a wide range of nonhuman primate (NHP) species and humans occupationally exposed to NHPs. Because of the limited antigenic cross-reactivity among some SFV variants, current serologic screening involve separate Western blot (WB) testing by different SFV antigens (SFVAGM and SFVCPZ). This SFV testing is labor intensive and the validation of the currently used assays is limited to a few NHP species. We developed a WB assay that combines both SFVAGM and SFVCPZ antigens in a single test. The combined antigen WB (CA-WB) assay was validated with serum samples from 128 primates (93 PCR-positive; 35 PCR-negative) representing 12 African and Asian primate genera and from 6 humans previously confirmed to be infected with SFV of baboon (n=4), chimpanzee (n=1) and African green monkey (n=1) origin. The CA-WB results were positive for all 6 SFV-infected humans. The sensitivity and specificity of the CA-WB was determined to be 98.9% and 97.1%, respectively. Cross-reactivity to CA-WB antigen was not observed in sera (n=25) from persons infected with HIV-1/2 or HTLV-1/2 nor in all 100 HIV- and HTLV-negative samples from U.S. blood donors. Using the CA-WB assay, we screened 319 captive NHPs from 24 North American zoos and 58 wild-caught Nigerian monkeys. 228 (71.5 %) of 319 captive NHPs and 17 (29.3%) of 58 of wild-caught NHPs tested positive. Overall, the results demonstrate the high sensitivity and specificity of the CA-WB in diagnosing SFV infection in humans and NHPs infected with divergent SFV.

1.10 Experimental Infection of Tamarins and Marmosets with Foamy Virus

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To address the potential of disease development associated with the cross-species transmission of foamy virus to a naïve host, an experimental infection study was initiated in nonhuman primates. We experimentally inoculated two New World monkey species, common marmosets (*Callitrix jacchus*) and cotton top tamarins (*Saguinus oedipus*) with chimpanzee foamy virus, SFVcpz.

Tamarins were selected because they have a limited MHC class I polymorphism, while marmosets are limited in their MHC class II repertoire. This limited potential to raise a specific immune response makes each species extremely susceptible for various viral infections. Therefore, we anticipated that these species may be good 'indicator species' for the detection of pathogenicity related to foamy virus infections.

From each species, four individuals were intravenously inoculated with a cell-free virus inoculum produced on a chimpanzee B-cell line, while four others were intramuscularly injected with foamy virus-infected B-cells. Blood samples and mouth swabs were taken every two weeks after inoculation for serology, virus isolation, and PCR analysis, as well as for hematological analyses and FACS analysis of specific cell subsets.

Four out of 8 marmosets and 5/8 tamarins became PCR-positive on DNA isolated from PBMC, but no virus could be recovered from the cultures of mouth swabs during the 6-month observation period. One of the infected marmosets, Mi080, died 8 weeks after infection, and tissue samples were obtained post-mortem. The remaining infected animals were euthanized 6 months after experimental inoculation. Tissue samples were taken for PCR analysis and histopathological examination. Clear differences were seen between the individual animals. The only tissues that were positive in all four marmosets were the axillary lymph nodes. Tissues that were positive in 3/4 marmosets were the spleen, lung, and the mesenteric lymph nodes. The data suggest a tropism of the virus for lymphoid and lung tissue. Histopathological analysis revealed interstitial pneumonia as the main finding in all four animals.

Five tamarins were positive at several time points post-infection. Six months post-infection the anaesthetized animals were perfused with saline, followed by a necropsy. All tissue samples are being processed for histopathologic and for PCR analysis. Details of the pathologic lesions and the virologic followup of SFVcpz infection in both species will be presented.

1.11 Infectivity Studies of Naturally Occurring Simian Foamy Viruses Isolated from Pig-tailed Macaques (*M. nemestrina*) and Rhesus Macaques (*M. mulatta*)

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To investigate SFV infection and latency in humans, we have initially studied the biological properties of naturally occurring SFVs that were isolated from rhesus (SFV-Mm) and pig-tailed (SFV-Mn) macaques by limited in vitro co-culture (<5 passages) of monkey peripheral blood mononulear cells and Mus dunni cells. The replication kinetics of SFV-Mm and SFV-Mn were investigated in various non-primate and primate cell lines, especially different human cell types, by monitoring cytopathic effect and reverse transcriptase activity. The results of the infectivity studies indicated that while prototype SFV serotype 1 (SFV-1) or serotype 2 (SFV-2) could replicate in all cell lines, latent infection of some human cell lines was seen with SFV-Mm and SFV-Mn isolates. Furthermore, SFV-Mm and SFV-Mn had slower replication kinetics than SFV-1 and SFV-2, and in general the order of replication efficiency was maintained in the different cell lines. These results indicate that naturally occurring SFVs have distinct biological properties as compared with the widely studied prototype SFVs, which may contribute to latent cross-species infection in humans. Investigation of factors involved in SFV latency in human cells will be discussed.

Session 2: Virology-Replication

2.1 Analysis of the Feline Foamy Virus (FFV) Replication Strategy

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The feline foamy virus (FFV) is member of the foamy virus (FV) subfamily of Retroviridae. Previous studies on the particular strategy of FV replication relied on the analysis of primate FVs, in particular PFV-1. Since there are some dissimilarities between FFV and PFV, e.g., in the respective Gag proteins, we wished to analyze whether the PFV-specific way of replication also applies to a more distantly related FV.

To do this, we constructed and characterized an infectious molecular clone for FFV. Furthermore, gag-pol expressing FFV vectors were generated and studied with respect to pseudotyping by various FV and non-FV Env proteins, sensitivity to the inhibition of reverse transcription by AZT, the presence of DNA vs. RNA in the extracellular virion, and the ability to retrotranspose intracellularly. The results of these studies will be presented.

2.2 Specific Interaction of a Novel Foamy Virus Env Leader Protein with the N-Terminal Gag Domain

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The ultra-structure of purified human and feline foamy virus (HFV and FFV) particles was analyzed by cryo-electron microscopy (cEM). The structural comparison revealed distinct radial arrangements of Gag proteins within released particles which have not been detectable by conventional EM techniques. The angular capsids were surrounded by an internal Gag protein layer that in turn was surrounded by, and separated from, the viral membrane. The width of this novel FV MA layer was about 8 nm for HFV and 3.8 nm for that of FFV. This difference in width of the FV MA layer is assumed to reflect the different sizes of the HFV and FFV MA domains: the HFV MA domain is about 130 residues longer than that of FFV. In FV particles with a regular morphology of properly budded particles, the MA layer closely followed the shape of the viral capsids. A close association of the MA layer with the viral membrane known from other retroviruses was not detectable in released FV virions. However, FV particles with a distended envelope and thus resembling budding intermediates displayed an invariant, close spacing between the MA layer and the Env membrane which was absent in the majority of particles. This indicates a specific interaction between MA and the viral membrane during a defined step of morphogenesis. This conclusion was supported by surface plasmon resonance studies. The purified N-terminal domain of FFV Gag expressed in bacteria specifically interacted with a 30-mer peptide derived from the N-terminal Env leader protein called Elp, whereas defined alterations in the Env peptide reduced or completely abolished binding. By genetic analysis, the corresponding sequence of HFV Env has been shown by Dirk Lindemann to be required for particle budding. The N-terminal FFV Env residues capable of interacting with Gag are part of the unprocessed Env precursor in FFV-infected cells. In released FFV particles, this motif is contained within the novel virion-associated FFV Env leader protein of about 16.5 kDa.

2.3 Domains of Foamy Virus Gag Involved in Particle Assembly and Morphogenesis

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Foamy virus (FV) capsid assembly occurs in the cytoplasm of infected cells in a manner similar to the B and D-type viruses. Recent evidence from experiments with the Mason-Pfizer monkey virus (M-PMV) indicates that intracellular assembly is mediated by a domain, termed the cytoplasmic targeting / retention signal (CTRS), involved in directing Gag to a discrete location within the cell to achieve the concentrations required for Gag-Gag interactions and capsid assembly. Alignment of all FV isolates reveals a consensus domain with moderate homology to the CTRS of MPMV. We found that mutation of an absolutely conserved arginine (Arg) residue at position 50 to alanine (R50A) of the simian foamy virus SFVcpz(hu) inhibits proper capsid assembly, as assessed by velocity sedimentation gradients (VSA), and abolishes viral budding. Virus assembly and extracellular release were restored to this mutant upon addition of an N-terminal Src-myristylation signal (Myr-R50A), presumably by providing an alternate site for assembly to occur at the plasma membrane (PM). Interestingly, Gag cleavage is completely inhibited in the Myr-R50A virus, and experiments are underway to quantitate levels of the Pol as well as nucleic acid in these particles. These studies should provide evidence for the site-specificity of FV assembly and may shed light on the mechanism of Pol incorporation.

In addition, the strict requirement of Env expression for capsid budding can be bypassed by addition of a PM targeting signal to Gag. The necessity of Env expression for particle egress is most likely due to the lack of an independent membrane-targeting signal within FV Gag. We are currently working to define the domains of Gag involved in the interaction with Env during viral budding. We have constructed a series of Gag deletion proviruses and have found a number of regions located at the amino-terminus of Gag that are required for capsid budding. Further experiments will determine whether there are separate Gag domains involved in particle assembly and release.

2.4 Complex Regulation of the Two Foamy Virus Promoters in Lytic and Persistent Infection

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The foamy virus (FV) genome contains two promoters, the canonical LTR promoter, containing three consensus AP-1 binding sites, and an internal promoter within the *env* gene. We have investigated the regulation of the two promoters in lytic and persistent infections and found that in the presence of a constitutive source of the transcriptional transactivator Tas, transactivation of the LTR and IP differ. In lytic infections, both the LTR and IP are efficiently transactivated by Tas. In persistent infections, the IP is efficiently transactivated by Tas and the LTR is transactivated to a lesser extent. Analysis of proteins expressed from the LTR and IP during infection indicate that IP transcription is more robust relative to the LTR in persistently infected cells, while the opposite is true in lytically infected cells. We also found that induction of persistently infected Jurkat cells with phorbol 12-myristate 13-acetate (PMA), or anti-CD3 and anti-CD28 antibody, greatly enhanced viral replication and transcription from the SFVcpz(hu) LTR while transcription from the IP was modestly augmented. Mutation of three consensus AP-1 binding sites in the FV LTR did not affect virus replication in lytically or persistently infected cells, nor did the same mutations affect LTR transactivation by Tas in PMA-treated cells. Furthermore, mutation of three putative ETS-1 binding sites alone, or in combination with the AP-1 binding site mutations, did not affect virus replication. Our data indicate that differential regulation of transcription is important in the outcome of FV infection but is unlikely to depend on AP-1 or ETS-1. Treatment of cells with a protein synthesis inhibitor at various times following PMA treatment indicates that de novo protein synthesis is required at all stages following PMA induction. Transient overexpression of Tas in persistently infected Jurkat cells can result in a switch to lytic infection; however, the levels of virus production are lower than observed with PMA treatment. We have recently identified additional cell types in which persistent FV infection can be activated by PMA treatment. These data are consistent with the presence of an inhibitor of FV replication in persistently infected cells. Recently, the promyelocytic leukemia protein (PML) has been identified as an inhibitor of FV replication in cells which support lytic replication. We are currently evaluating the role of PML in limiting FV replication in persistently infected cells.

2.5 The Highly Conserved Sequence 'TGTG' at the 5'end of U3 in Foamy Virus Genomes

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Integration is an essential step in the replication cycle of retroviruses. Mutations that interfere with integration block orthoretroviral and foamy viral replication. Integration contributes to viral replication in two important ways. First, retroviral gene expression depends on integrated viral genomes. Second, since retroviral DNA molecules are not ordinarily able to replicate autonomously as episomes, they depend on integration for stable maintenance in dividing cells. Once integrated, however, the provirus is replicated along with host-cell DNA and genetically transmitted as an integrated element of the host genome. Integration in foamy viruses appears to diverge from orthoretroviral integration since a highly conserved 'TGTG' motif at the 5'end of U3 is not processed during integration, while the 3'end of U5 is readily cleaved.
To study the function of the highly conserved 'TGTG' (ppt-U3 border), we created a variety of mutants in this sequence. These mutants were compared to wild-type virus with respect to viral replication, gene expression and particle release, subcellular localization of Pol proteins, and the formation of circular forms of the viral DNA following transfection and infection. The results of these experiments will be presented.

2.6 Centrosomal Targeting of HFV Requires the Interaction of the Structural Gag Protein with LC8, the Cytoplasmic Light Chain of Dynein

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Many intracellular pathogens have evolved specific mechanisms to exploit the host cell systems to their own profit. Viruses such as vaccinia virus or herpes viruses use the microtubule (MT) network to reach their site of replication. However, little is known on the role of the cytoskeleton on the replication of retroviruses. We have previously shown that the viral cycle of foamy viruses (FVs), which are animal complex retroviruses, requires an intact MT network. In the early steps of infection, the viral genome reaches the microtubule organizing centre (MTOC) prior to nuclear translocation (Saïb A, et al. 1997). Here, we demonstrate that the structural Gag protein of HFV, the prototype of FVs, is able, out of a viral context, to target the MTOC in transfected cells. This transport is inhibited by a treatment with nocodazole or in cells over-expressing p50/dynamitin, demonstrating the involvement of dynein, a retrograde MT-associated motor, in MTOC targeting. This interaction was confirmed by co-localization and by co-immunoprecipitation. Taken together, these results provide for the first time a molecular basis of intracellular trafficking of a retrovirus during the early steps of infection.

2.7 Identifying Bel1-Activated Human Genes and Transcriptional Regulation of Human cdkn1c Gene by the Spumaretroviral Bel 1

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Expression of the human cyclin-dependent protein kinase inhibitor p57Kip2 gene (cdkn1c) was previously shown to be specifically activated by the retroviral *trans*-activator Bel 1 (also called Tas) of human foamy virus (HFV) by means of expression profiling. RNA blot analyses showed a gene-specific and time-dependent induction compared with mock-infected controls. Western blot analyses with monoclonal antibodies against distinct Bel-1 activated human genes confirmed the results of the expression profiling that had been done with cDNA array assays (A. Wagner et al. J. Virol. 74, 4441-4447, 2000). Electrophoretic mobility shift and supershift assays in combination with reporter asays were performed with the p57kip2 gene. The analysis revealed that p57Kip2 binds the bel1 response element (Kip2-BRE) directly and that this DNA target site is located at an intragenic position of the p57Kip2 gene. In direct comparative experiments, the viral internal promoter was used as control.

In addition to p57Kip2, various other cellular genes were activated by Bel1 transfection and HFV infection. The celluar genes that were induced by HFV infection and after transfection with bel1 included the human fetal growth factor and oncogene IGF-II, transcription factors EGR-1, and COUP-TF1, and the Tyrosine protein kinase receptor EphB3. A cellular BRE consensus sequence was derived from Kip2-BRE and those human genes specifically activated by bel1 including insulin-like growth factor and several transcription factors.

A paradox between the well-known pathogenic properties of p57Kip2 and IGF-II genes that were expressed after HFV infection and the apparently apathogenic consequences of an HFV infection will be discussed.

2.8 Transcriptional Regulation of the Human p57Kip2 Gene by Sp1 and the Spumaretroviral *Trans*-activator

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Expression of the human cyclin-dependent protein kinase inhibitor p57Kip2 gene was previously shown to be specifically activated by the retroviral *trans*-activator Bel1 (Tas) of human foamy virus (HFV) by means of expression profiling and Northern and Western blot analysis. Here, we report that Bel1-mediated *trans*-activation is conferred by a 55-nucleotide-long DNA element (Kip2-BRE) located in the first coding exon of p57Kip2. This intragenic Bel1 response element, Kip2-BRE consists of three direct repeats of 15mers homologous to a functionally active BRE in the internal promoter of the infectious HFV provirus previously shown to be capable of directly binding the Bel1 protein (Kang et al. J. Virol. 72, 504-511, 1998). The viral BRE and the Kip2-BRE site contain a completely conserved G base pattern.
Bel1 has the capacity to interact with cellular transcription factors. This interaction was detected when the influence of the transcription factor Sp1 on the function of Bel1 was analyzed. Under the conditions used, overexpression of Sp1 alone did not show any activation of the p57Kip2 promoter, although many Sp1 binding sites were identified in the promoter sequence. In the presence of Bel1, however, Sp1 synergistically enhanced the Bel1-mediated *trans*-activation. As Sp1 binding sites had to be present in the authentic and chimeric p57Kip2 promoters, we assume that Sp1 was recruited to the promoter by its intrinsic DNA binding ability. The requirement of an appropriate spacing of the Sp1 sites relative to those of BREs and the presence of binding sites of additional transcription factors might be responsible for the significantly stronger Sp1 effect in the authentic p57Kip2 promoter versus the chimeric SV40-p57Kip2 promoter used in some of the expression constructs studied here. Mutagenesis of distinct nucleotides within the Kip2-BRE site will further delineate the precise DNA target site of Bel1. Our data characterize the p57Kip2 promoter and indicate that Sp1 has a role in *trans*-activating Kip2-BRE.

2.9 Inter- and Intracellular Movements of the Accessory Foamy Virus Bet Protein

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The accessory Bet protein is one of the most intriguing foamy virus proteins. Highly expressed during infection, Bet has been implicated in viral persistence (Saïb *et al.*, 1995). Moreover, cells expressing this protein were shown to be resistant to productive HFV infection (Bock *et al.*, 1999). To shed a new light on the functions and mechanism of action of Bet, we have analyzed its sub-cellular distribution.

We have already shown that Bet was secreted by transfected cells and internalized by surrounding naïve ones (Giron *et al.*, 1999). Here, we show, by confocal microscopy analysis, that Bet is detected both in the cytoplasm and the nucleus of HFV-infected or Bet-expressing cells. This sub-cellular localization was further confirmed by biochemical analysis. The nuclear localization of HFV Bet results from the presence of a bipartite nuclear localization signal at the C-terminus of the protein, allowing the nuclear targeting of the GFP reporter. To see whether these properties are conserved among FVs, the Bet protein from the distantly related equine foamy virus (EFV) was cloned and its sub-cellular localization was analyzed. This study revealed that these two features (inter-cellular movements and nucleo-cytoplasmic distribution) were also observed for EFV Bet.

We are currently addressing whether these characteristics are involved in resistance to FV superinfection, either in Bet-expressing cells or in those which have internalized Bet.

2.10 Pregenomic RNA is Required to Incorporate Pol into Foamy Virus Capsids

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In all non-foamy virus (FV) retroviruses (orthoretroviruses), the Pol protein is expressed as a Gag-Pol precursor protein. The cotranslation of Pol by suppression of a *gag* stop codon or -1 ribosomal frame shifting is generally accepted to serve two functions: (i) it guarantees a relatively fixed ratio of Gag to Pol, thus structural to enzymatic proteins, of 10-20:1, and (ii) it enables the incorporation of Pol protein into the viral capsid. The FVs *pol* ORF is not translated together with *gag*, since it is essentially expressed from a spliced mRNA. Therefore, the rules of orthoretroviral Pol incorporation do not apply to FVs. There are principally three ways how Pol protein can be assembled into FV capsids: (i) protein-protein interactions with Gag may be essential and sufficient for Pol incorporation, (ii) alternatively this may be facilitated by an interaction between Pol and the pregomic RNA, or (iii) a combination of both may bring Pol into the viral particle. Using a novel transient FV vector transfection system, it is shown that pregenomic RNA is required for virion incorporation of functionally active Pol and that protein-protein interactions of Pol with Gag are not sufficient to complete FV particle assembly. Thus, we suggest RNA as a bridging molecule to assemble a functionally active FV capsid.

2.11 Decrease in Processivity of a Reverse Transcriptase Catalytic-site Mutant Blocks Foamy Virus Replication

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Foamy viruses (FVs) are classified as complex retroviruses; however, they differ from conventional retroviruses in important ways. A notable difference is in the timing of reverse transcription. FV particles contain both RNA and DNA. Most of the reverse transcription reaction occurs in the cells that produce virions. Thus, DNA appears to be the functional viral genome. The mechanism of Pol expression and the incorporation of the Pol polyprotein into the viral particle are also different. Instead of a typical Gag-Pol fusion protein, FV Pol is expressed from its own message. While the exact mechanism of Pol packaging is unclear, the Pol may bind specifically to the genomic RNA. This would lead to the incorporation of only 1-2 Pol proteins per virion, consistent with the inability to detect Pol in particles by Western blotting.

HFV replication is resistant to most nucleoside analogs. To create a reverse transcriptase (RT) that is sensitive to 3TC, the second residue in the highly conserved YXDD motif was changed from V to M. The resulting virus, HFV RT-V319M, replicated poorly, and the YMDD motif rapidly reverted to YVDD. Although RT-V319M virions contain about 60% of the RT activity found in wild-type virions, full-length DNA products are not detected in transfected cells. Using purified recombinant RTs, we found that the V319M mutant has about 50% of the wild-type level of RT activity. Wild-type HFV-RT is a highly processive enzyme; RT-V319M is less processive than wild-type RT. This suggests that the decrease in processivity affects the ability of HFV RT-V319M to complete reverse transcription.

2.12 Characterization of FV Isolates after AZT-resistance Induction

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Retroviruses such as HIV can develop drug resistance during anti-viral treatment with nucleoside analogs. This phenomenon depends on mutations within the reverse transcriptase (RT) coding region of the pol gene eventually leading to alterations of the secondary structure. We analyzed the in vitro ability of FV to develop resistance against the anti-retroviral nucleoside analog AZT. After resistant FVs had been established, mutations within the polymerase gene were analyzed. Mutations were introduced into infectious molecular clones to verify the observation.

Full-length molecular clones of SFVcpz, SFV-1, FFV, and PFV-1, the isolate previously known as HFV, were used to produce infectious virus supernatants after transfection of 293T cells. BHK-21 or CrFK cells, respectively, were infected in the presence of AZT. Generally, at 0.05 µM of AZT, FV replication was abrogated. At lower AZT concentrations ranging from 0.001 to 0.005 µM, FV could be propagated at very low levels. As determined by assaying culture supernatants on indicator cells expressing LacZ under control of the corresponding LTRs, SFV-1 and FFV that had been maintained in the presence of 0.001 µM AZT for some time could also resist 0.01 µM AZT. After transfer of supernatants and successive slight elevation of AZT concentrations to 0.1 µM, these viruses kept slowly replicating. Throughout several rounds of infection, minimal replication of the latter viruses could be maintained when AZT concentrations were successively increased by small increments. After approx. 70 days both SFV-1 and FFV resisted 1 mM of AZT and yielded virus titers comparably to those of the parental viruses. PCR amplification and analysis of FV proviral DNA showed mutations altering amino acids located approx. 30 amino acids C-terminal of the RT's active center. Interestingly, most mutations in HIV-1 conferring AZT resistance are located within a corresponding region of the HIV-RT. We introduced the mutations found in AZT-resistant SFV-1 into the parental clone as well as into pcHSRV2, which carries infectious DNA of PFV-1. While pSFV-1-AZT gained the ability to replicate in the presence of 1 mM AZT, pcHSRV2-AZT remained sensitive to the drug.

2.13 Foamy Virus Infectivity is Inhibited by Lysosomotropic Agents

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Little is known about the first steps of the Foamy Virus (FV) replication cycle, namely binding to the cellular receptor, fusion of viral and host cell lipid membranes, and entry of the viral capsid into the target cell. The fusion process of most other retroviruses has been shown to occur at the plasma membrane and is largely pH-independent. However, only recently a pH-dependent uptake of Avian Sarcoma Leukosis Virus (ASLV) has been described and ecotropic Murine Leukemia Virus (MuLV) envelope mediated infection is pH-dependent in a cell type specifc manner.
In order to gain more information about the FV Env mediated fusion process leading to the cellular uptake of viral capsid structures, we analyzed the infectivity of MuLV vector pseudotypes with different viral envelope glycoproteins in the presence of various lysosomotropic agents. These agents reversibly increase the pH of intracellular compartments, thereby inhibiting endocytosis mediated, pH-dependent fusion processes. The amphotropic MuLV Env and the Vesicular Stomatitis Virus glycoprotein G (VSV-G) were used as controls for a pH-independent and pH-dependent fusion process, respectively. MuLV retroviral pseudotype supernatants expressing an EGFP marker gene were generated by transient transfection of 293T cells. Subsequently, a pretitrated amount of pseudotypes was used to infect different target cells in the presence of varying concentrations of different lysosomotropic agents. Forty-eight hours later, the percentage of EGFP expressing target cells was determined by FACS analysis and the effect of the lysosomotropic agents was expressed as relative infectivity compared to cells infected with vectors alone. FV Env mediated infectivity was inhibited by ammonium chloride, methylamine, concanamycin A, and bafilomycin A1, but not by chloroquine. FV vectors and replication-competent FV showed a similar inhibition of infectivity by the above-mentioned substances. Our results point to a dependence of FV Env mediated fusion on vacuolar ATPases, that are specifically inhibited by concanamycin A and bafilomycin A1. The inhibition by other lysosomotropic agents but not by chloroquine may point to an endocytose-depependent fusion process that is different from that of other viruses.

Session 3: Foamy Virus Vectors

3.1 Transduction of SCID Repopulating Cells by a Human Foamy Virus Vector

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We have developed a gene transfer system based on the Human Foamy Virus (HFV). Using transient transfections of 293T cells, we can produce stocks with titers of 1-5 x 10^5 transducing units/ml that are free of replication-competent retrovirus. These stocks can then be concentrated 100-fold by centrifugation. We used HFV vectors to transduce human repopulating cells in the NOD/SCID xenotransplantation model. In four separate experiments, we transplanted a total of 16 sub-lethally irradiated mice with transduced human umbilical cord blood CD34+ cells. Transductions were performed for 10 hours in CH-296 fibronectin fragment coated dishes with an HFV vector containing the enhanced green fluorescent protein reporter gene (GFP). At 5-7 weeks post-transplantation human hematopoietic cell engraftment into recipient marrow and marking rates were determined by flow cytometric analysis. The mean engraftment rate with human CD45+ cells was 21.0 ± 19.8 %, and these cells exhibited a GFP expression rate of 49.2 ± 20.7 %. Similar levels of GFP expression could be detected in human CD19+ (lymphoid), CD33+ (myeloid), and CD34+ cells. Furthermore, marking in human clonogenic progenitors derived from transplanted mice was 62.5 ± 26.7%.

We performed vector integration site analysis by Southern blotting of DNA made from polyclonally expanded human myeloid and immortalized human lymphoid populations derived from transplanted mice. We found multiple different proviral integration patterns within the repopulating cells of each transplanted mouse studied. A transgene copy number of up to 3.8 was seen in the expanded GFP+ myeloid cells. Furthermore, some of the integration sites found in lymphoid populations were also demonstrated in expanded myeloid cells, indicating that a hematopoietic stem cell capable of myeloid and lymphoid differentiation had been transduced.

Our study demonstrates that HFV vectors are able to efficiently transduce human hematopoietic cells capable of repopulating NOD/SCID mice, including cells with pluripotent capacity. These findings indicate that HFV vectors are a promising gene delivery system for use in hematopoietic stem cell gene therapy.

3.2 Transduction of Hematopoietic Stem Cells (HSCs) with Human Foamy Virus (HFV) Vectors

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Vectors based on foamy viruses (FVs) have advantages over other retroviral systems: a larger packaging capacity and a complete absence of disease-association. Self-inactivating FV vectors have been produced by triple co-transfection to minimize the generation of replication-competent retroviruses. Vectors packaging the marker genes *LacZ* or enhanced *GFP* have been produced in 293T cells at titers of 10^5 to 10^6 transducing units/ml. The vector stocks were concentrated by ultracentrifugation to 10^6 to 10^7 transducing units/ml for transduction of HSCs. The optimum titer obtained to date is 10^8 transducing units/ml. FV and murine leukemia virus (MLV) vectors have been used to transduce $CD34^+$ HSCs from cord blood to compare their transduction efficiencies. Transduction times were minimized to reduce exposure of HSCs to cytokines *ex vivo*, in order to optimize engraftment in NOD/SCID mice. The $CD34^+$ HSCs were transduced on RetroNectin with equivalent titers of HFV and MLV vectors within 24 hours of isolation from cord blood. Transgene expression in hematopoietic progenitor cells, analyzed by microscopy or flow cytometry after 3-4 days culture *in vitro* with cytokines, was consistently six- to ten-fold greater with the FV vectors than with MLV vectors. Similar results were seen in their differentiated progeny after 14 days in methylcellulose-based culture. HFV vectors expressing eGFP are being used to transduce SCID repopulating cells in NOD/SCID mice and the long-term expression *in vivo* in mouse bone marrow monitored after 6 weeks. We aim to investigate ways of upregulating transgene expression. For example, we will replace the spleen focus-forming virus (SFFV) U3 promoter driving this expression with others known to perform well in HSCs. Additionally, we shall introduce the Woodchuck Hepatitis B Post-Transcriptional Element into the vector constructs.

3.3 Efficient Transduction of Human CD34+ Cord Blood Cells with a Primate Foamy Virus Vector

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Hematopoietic stem cells (HSCs) are attractive targets for gene therapy of inherited hematopoietic disorders because corrected stem cells have the ability to regenerate the entire hematopoietic system. Efficient retroviral transduction of HSCs with oncoretroviral vectors based on the murine leukemia virus (MLV) is only possible if cells have been prestimulated to divide by early acting cytokines for extended periods of time. Since foamy viral vectors might be able to transduce nondividing cells, we investigated whether they can be utilized to genetically modify human CD34+ cord blood (CB) cells.
Recombinant primate foamy virus (PFV-1) and gibbon ape leukemia (GalV) envelope pseudotyped MLV containing the same EGFP expression cassette were produced in a transient two plasmid packaging system. Retroviral supernatants had titers of $1 – 5 \times 10^7$ transducing units/ml. Human CD34+ CB cells were transduced overnight in the presence of SCF, TPO, and G-CSF on CH296-coated plates, either immediately after MACS selection or after a two-day prestimulation with the same cytokine cocktail. Transduced cells were plated in standard progenitor assays, and the percentage of EGFP+ colonies was scored at day 14 by fluorescence microscopy.
Results: While transduction efficiencies of prestimulated CD34+ cells were similar between MLV and PFV vectors (69.2±6.1% versus 72.4±7.5% EGFP+ colonies), efficient transduction of unstimulated cells was only possible with the PFV vector. The percentage of EGFP+ colonies transduced with the PFV vector was 11-fold higher than that of MLV transduced colonies (70.1±7,8% versus 6.1±1.2% EGFP+ colonies).
Conclusion: Our results show a high potential of PFV-based vectors for use in HSC gene therapy, since efficient transduction of colony forming progenitor cells is possible with a short *ex vivo* manipulation of human CD34+ cells.

3.4 Ex vivo Transduction by Foamy Virus-derived Vectors and Hepatic Reimplantation of Primate Hepatocytes

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There is no evidence that foamy viruses (FV) are pathogenic in either their natural hosts (non-human primates) or accidentally infected humans. Apathogenicity together with large genome size and broad host range make them ideal candidate vectors for somatic gene therapy. The liver is a preferred target for gene therapy since it is the major site of metabolic processes. Therefore, the aim of our study was *ex vivo* application of FV-derived vectors to hepatocytes isolated from resected tissue of African green monkey (AGM) donors. We have chosen *lac*Z to monitor gene delivery. Hepatocytes cultured from livers of rats, marmosets, and AGM were transduced with either supernatants or cell extracts of vector-producing 293T cells and analyzed for *lac*Z expression. On hepatocytes, titers of 10^2-10^4 transducing particles/ml were reached compared to 10^4-10^5 transducing particles/ml on fibroblastic cell lines such as BHK-21 or NIH3T3. An ex vivo gene transfer experiment in AGM included liver lobe resection and implantation of a portal vein catheter. The cultivated and transduced AGM hepatocytes were reinfused, 48 hours after in vitro transduction, into the regenerating liver of the donor animal via subcutaneous port. A few *lac*Z expressing hepatocytes were traced in biopsies taken one and two weeks thereafter. Thus, these preliminary studies have shown that FV-derived vectors are able to deliver genes into primate liver tissue. Although PCR on biopsy material has shown the presence of *lac*Z up to 6 weeks after reinfusion, it is obvious that the efficiency of foamy virus-derived vectors has to be improved, to allow therapeutically effective expression of a foreign gene in vivo.

3.5 Transduction Properties of Tas-Independent Foamy Vectors and Development of Improved Vectors with Minimal *Cis*-acting Regions

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Foamy vectors have several attributes, including a broad host range, no demonstrated pathogenicity, and a large genome, that make them attractive gene therapy vehicles. In addition, foamy vectors transduce stationary fibroblasts more efficiently than MLV vectors. Second generation helper-free foamy vectors can be generated at titers of 10^5/ml by transient transfection of 293T cells and further concentrated to 10^8/ml by ultracentrifugation and have shown promise for transduction of hematopoietic stem cells. We directly compared the transduction rates of these foamy vectors to HIV and MLV vectors in stationary human fibroblasts and found that foamy vectors were as efficient as an HIV vector that contained a cPPT. In addition, foamy vectors were able to efficiently transduce arrested cells that were then stimulated to divide up to 10 days after vector exposure, the longest time point tested. This suggests that foamy vectors form a stable transduction intermediate and will be useful for transducing stem cells that are quiescent during transduction but enter the cell cycle later. We also noted that transduction of both serum-starved human fibroblasts and rat 208F fibroblasts that were not released from arrest occurred only in cells that had undergone DNA synthesis as evidenced by BrdU staining. So, while foamy viruses are clearly superior to MLV vectors and similar to HIV vectors at transducing stationary cultures, there may still be factors present in proliferating (and especially S-phase) cells that further increase transduction efficiencies. These results suggest foamy vectors will be useful for therapeutic applications, however our current vectors retain the complete *gag* and *pol* genes. We measured the relative packaging efficiency of deleted vectors with wt HFV and identified a 2.4 kb *cis*-acting region that contained previously defined *pol cis*-acting sequences. We found that high titer vector stocks were necessary to detect subtle packaging requirements. A deleted foamy ($\Delta\Phi$) vector was constructed that contained this *cis*-acting region and also had a 582 bp deletion in the U3 region of the LTR that includes the TATA box. The $\Delta\Phi$ vectors can package 6.9 kb of foreign transgene at titers of $4X10^4$/ml and should be useful for *ex vivo* gene therapy.

3.6 Foamy Viruses as Live Vaccine Vectors for HIV

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Since retroviruses are capable of integrating their genome into host DNA, they provide an efficient means for introducing foreign DNA. However, the use of "integrating" viruses for gene therapy or vaccine approaches to treatment and prevention of human disease raises safety concerns. We evaluated the possibility of using foamy viruses as a vaccine tool to take advantage of the natural history of human and animal infections with these agents, which have shown no pathogenicity. Using the human foamy virus (HFV) infectious molecular clone developed by Rethwilm, we have engineered both HIV-1 env and gag genes into the pFOV vector and analyzed them for gene expression and stability in vitro. The pFOV clone was provided containing GFP in place of the bet 2 gene. Either pFOV 10 (containing an SFFV U3 promoter) or pFOV 7 (containing only an internal promoter) were used to insert HIV-1 env (gp160) or HIV-1 gag (p17-p24), respectively. Results showed that pFOV 10-env produced gp120, as detected by a CD4-binding assay, only transiently after transfection into BHK cells. Further analysis showed the gp160 (approx. 3.0 kb) truncated and abrogated expression prior to foamy virus replication. The pFOV HIV gag construct, however, produced high levels (up to 1 ug/ml) of p24 antigen as detected by p24 antigen capture after transfection. Furthermore, the foamy virus recovered from the transfection could be passaged as infectious virus and retained the HIV gag gene along with p24 expression (>1 ug/ml on day 7 post infection). These data show that stable high-level expression of HIV gag can be produced in live, replicating foamy virus vectors.

3.7 Efficient Pseudotyping of Human Immunodeficiency Virus and Murine Leukemia Virus Particles with Foamy Virus Envelope Proteins

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Vectors derived from human immunodeficiency virus (HIV) and murine leukemia virus (MLV) are frequently applied as vehicles for gene transfer. These vectors are used in combination with a variety of different envelope proteins with the intention to alter the tropism and achieve selective or increased infection efficiencies on specific target cell types. We have described previously the pseudotyping of MLV vectors with chimeric foamy virus (FV) envelope proteins. The aim of this study was to further improve MLV pseudotyping titers and define FV Env proteins capable of pseudotyping lentiviral vectors.

We have studied the infection efficiencies of HIV and MLV vector particles pseudotyped with wild-type and chimeric FV envelope proteins from virus isolates derived from different species. Pseudotyping of both vector types with different FV envelope proteins was achieved. Using a transient transfection vector production system, the best results were obtained with wild-type feline foamy virus (FFV) envelope pseudotypes having titers of up to 105 ffu/ml on 293 target cells, about one log lower than those of pseudotypes with the VSV-G protein.

In conclusion, lentiviruses such as HIV and MLV can now be efficiently pseudotyped with FV envelope proteins. As a result of some unique properties of FVs, pseudotyping of these vectors with the FV envelope might provide a useful tool for different gene transfer approaches.

3.8 Circular DNA with Two Tandem LTRs Does Not Serve as a Target for Integration by the HFV Integrase

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The presence of closed circular forms of the linear DNA genome of human foamy virus (HFV) has not been established. The ability of the HFV integrase (IN) to catalyze the integration of these circular forms (termed 2 long terminal repeat [LTR] circles) was investigated, with a view of producing a novel hybrid vector. To do so, a construct was made containing, in addition to the enhanced green fluorescent protein (*eGFP*) marker gene, the last 27bp of the 3' U5 LTR region of HFV fused to the first 28bp of the 5' U3 LTR, the latter representing a 2LTR circle. Marker gene expression following transfection of both 293 and 293T cells indicated that the level of integration was not significantly increased by the HFV IN. Moreover, correctly integrated provirus-like forms of the input plasmid could not be detected by PCR. Taken together, these results show that the HFV IN is not able to integrate a circular molecule containing an LTR junction and, hence, the technique is not exploitable as a tool to produce novel vectors for gene therapy.

3.9 The *bet* Gene of Feline Foamy Virus Is Required for Virus Replication: Implications for FFV Vector Construction

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The functions of the foamy virus (FV) *bel 2* and *bet* genes are unknown and both are almost dispensable for replication of the prototypic human FV HFV in cell cultures. We examined the function(s) of *bel 2* and *bet* on the replication of the distantly related feline foamy virus (FFV). Site-directed mutagenesis was used to alter Bel 2 and Bet or to abrogate their expression. The Bel 2/Bet mutants had a 1000-fold reduced infectivity in feline kidney cells; in human 293T cells, viral infectivity was only about 10-fold reduced when compared to wild-type FFV. In both cell types, the Bel 2/Bet mutations resulted in a reduced release of FFV particles. The results indicate that FFV Bet is required for efficient virus replication.

Based on these data, replication-competent FFV-based vector genomes were constructed in which heterologous proteins were fused in frame to FFV Bel 2/Bet. The replication kinetics of these chimeric viruses was comparable to that of wild-type FFV, however, the foreign sequences were deleted upon longer vector propagation. Analyses of these revertants revealed that Bel 2/Bet was retained intact, whereas almost the complete heterologous protein sequences had been deleted. This result supports our notion that an intact *bel 2/bet* gene is required for FFV replication in vivo. Potential functions of the Bel 2 and Bet proteins will be discussed.

3.10 Analysis of Foamy Virus Gag and Pol Expression Plasmids for Packaging of Foamy Virus Gene Transfer Vectors

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Due to various advantageous features there is current interest in retroviral vectors derived from primate foamy viruses. Improved foamy virus (FV) vectors require packaging plasmids for *trans*-complementation of the structural virus proteins. First generation packaging constructs for Gag and Pol expression harbour the complete 5' untranslated region (UTR) of the FV genome. We previously analysed Gag/Pol expression plasmids with deletions in the 5' UTR. We reported an element within the R region which is essential for FV Gag and Pol expression. No packaging signal could be detected within the 5'UTR, and RNA transcribed from Gag/Pol expression plasmids harbouring large deletions in the 5'UTR was readily packaged into FV vector capsids. The results of our present study indicate that the FV splice donor (SD) is the main element within the R region which is required for Gag and Pol expression. We designed several Gag and Gag/Pol expression constructs devoid of FV 5' UTR sequences by insertion of heteologous SD or intron elements upstream of the gag ATG. These constructs were analysed for protein expression, vector transfer efficacy, and for packaging of the RNA transcribed from the packaging system into the vector particles. In these experiments, two Gag expression constructs for efficient generation of capsids containing little or no RNA transcribed from the packaging constructs were identified.

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